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# Airbone Laser Fluorosensing of Surface Water Chlorophyl 'a'

**Environmental Monitoring and Support Lab, Las Vegas, NV**

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Environmental Monitoring
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# Airborne Laser Fluorosensing of Surface Water Chlorophyll a

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16. ABSTRACT

A prototype airborne laser fluorosensor for monitoring surface water chlorophyll a has been tested over Lake Mead, Nevada. Trends in the remotely sensed data are in close correspondence with ground truth data. It is suggested that system performance can be improved by concurrently gauging the water optical attenuation coefficient and by implementing changes to the established procedure for performing chlorophyll a analyses on ground truth samples.

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# AIRBORNE LASER FLUOROSENSING OF SURFACE WATER CHLOROPHYLL a

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# FOREWORD

Protection of the environment requires effective regulatory actions that are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach that transcends the media of air, water, and land. The Environmental Monitoring Systems Laboratory-Las Vegas contributes to the formation and enhancement of a sound monitoring data base for exposure assessment through programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report describes the development of an airborne laser fluorosensor for mapping the distribution of chlorophyll a in the surface waters of lakes, rivers, reservoirs, estuaries and coastal waters. Remote monitoring devices of this kind will be invaluable for rapidly assessing the concentration of surface water algae for a given water body and, in particular, for locating anomalies, gradients and blooms produced by anthropogenic activity. Further information concerning the development of this system can be obtained from the Advanced Systems Branch, Advanced Monitoring Systems Division.

George B. Morgan

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# SUMMARY

In fresh-water environments, high concentrations of chlorophyll a indicate the presence of high levels of planktonic algae. This condition suggests, in turn, the existence of eutrophic conditions, that situation in which high concentrations of nutrients create algal bloom conditions. Such conditions can lead to malodorous and even toxic water conditions. Conversely, anomalously low chlorophyll a levels might indicate the presence or influence of a source of toxic pollutants. Chlorophyll a is also important in the marine environment. As phytoplankton are a primary food source, their distribution and productivity are of considerable interest to the fishing industry.

A program has therefore been conducted to develop an airborne laser-fluorosensing device capable of directly mapping surface-water distributions of chlorophyll a without requiring empirical data-interpretation schemes derived from ground truth measurements.

A profiling airborne laser fluorosensor has demonstrated an ability to monitor general trends in relative surface water chlorophyll a distributions over the range from 2 μg/l to 20 μg/l at elevations from 200 m to 400 m under clear sky, daylight conditions. The laser fluorosensor in its present form is capable of monitoring chlorophyll a concentrations down to 0.4 μg/l with a minimum signal-to-background noise ratio of 3. With the implementation of measures to increase the fluorescence signal and reduce the background noise, the detection of chlorophyll a levels should be possible down to 0.1 μg/l with a minimum signal-to-background noise ratio of 20. Comparisons between chlorophyll a ground truth data and the airborne chlorophyll a fluorescence signal produced linear correlation coefficients in the range from 0.77 to 0.95.

It is anticipated that improvements in this degree of covariation can be accomplished by implementation of a number of measures. First, a need for more efficient and reproducible methods for making chlorophyll a ground truth determinations is indicated. Second, the laser-fluorosensor chlorophyll a fluorescence signal has been shown to be sensitive to changes in the water optical attenuation coefficient. A means for correcting for this effect by concurrently monitoring the water Raman emission signal has been proposed. Finally, it is suggested that tests be conducted to determine the optimum wavelength for exciting fluorescence from in vivo chlorophyll a contained in mixtures of algae from the different color groups.

Attempts to monitor changes in the surface water distribution of blue-green algae using the single wavelength excitation laser-fluorosensor technique were not successful.

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## LIST OF ABBREVIATIONS AND SYMBOLS

### ABBREVIATIONS

EMI -- Electromagnetic interference
f/number -- Ratio of focal length to diameter of optical system aperture
FLD -- Fraunhofer line discriminator
FWHM -- Full width at half maximum height
{OH} -- Notation for hydroxyl radical, as in water molecule
ppb -- Parts per billion
pps -- Pulses per second
r -- Pearsons product moment linear correlation coefficient
RFI -- Radio frequency interference
RMS -- Root mean square value
SBNR -- Signal to background noise ratio
s -- Sample standard deviation
UV -- Ultraviolet
$\bar{x}$ -- Sample mean
$\theta$ -- Laser beam divergence

### SUBSCRIPTS

b -- Backward scatter
c -- Chlorophyll a
f -- Forward scatter
F -- Fluorescence
L -- Laser
N -- Non-chlorophyllous material
pp -- Peak to peak
P -- Peak
R -- Raman
Rec -- Receiver
RMS -- Root mean square
Tr -- Transmitter
W -- Water
$\lambda$ -- Wavelength

## LIST OF ABBREVIATIONS AND SYMBOLS (continued)

### SYMBOLS

$a$ -- Absorption coefficient
$A$ -- Intercept constant in Equation 9
$b$ -- Scattering coefficient
$B$ -- Slope constant in Equation 9
$d$ -- Terms defined by Equations 16 and 18
$D$ -- Dimensionless radiance distribution factor
$H$ -- Target range
$k$ -- Water diffuse attenuation coefficient
$n$ -- Concentration
$P$ -- Peak power
$R$ -- Reflectivity
$T$ -- Effective area of telescope aperture
$v$ -- Noise signal voltage
$V$ -- Peak signal voltage
$x$ -- Optical path length
$X$ -- Optical attenuation terms, defined by Equations 13 and 14
$\alpha$ -- Water beam attenuation coefficient
$\beta$ -- Atmospheric attenuation coefficient
$\delta$ -- Constant in Equation 20
$\Delta$ -- Fraction of emission band seen by detector
$\eta$ -- System optical efficiency
$\mu$ -- Refractive index
$\sigma$ -- Excitation cross section

# SECTION 1

# INTRODUCTION

In fresh-water environments a high concentration of chlorophyll a indicates high levels of planktonic algae. This condition suggests, in turn, the existence of eutrophic conditions, that situation in which high concentrations of nutrients create algal bloom conditions. Such conditions can lead to malodorous and even toxic water conditions. Conversely, anomalously low chlorophyll a levels might indicate the presence or influence of toxic pollutants. Chlorophyll a is also of importance in the marine environment. Phytoplankton are the principle producers and suppliers of energy in the marine ecosystem. They are the principal food source for the herbivores such as zooplankton, which in turn are an important food source of the carnivores characterized by the fish population. As a consequence, information relating to phytoplankton productivity is of considerable interest to the fisheries industries.

Chlorophyll a determinations are routinely accomplished by laboratory analyses on grab samples. This approach is both time-consuming and costly in terms of manpower and facilities. In addition, because of the finite time required to take grab samples from launches or helicopters, it is not always possible to obtain a synoptic record for a given water surface due to water movement and diurnal effects. Also, delays in transporting water samples to the laboratory for analysis or the practice of freezing algae samples collected on filters are known to produce large errors due to irreversible degradation of the chlorophyll a. In contrast, both airborne and satellite remote-monitoring techniques are capable of rapidly and cost effectively providing data for certain water quality parameters from large areas of water surface without influencing the nature of the sample. The principal limitation of remote sensing is its inability to provide information from below the photic zone, commonly defined as that region from the surface to the depth at which 99% of the surface light has disappeared. For active as well as passive sensors operating in the optical region, this depth is generally on the order of 0.5 to 10 meters and is conveniently characterized by the reciprocal of the attenuation coefficient measured at a given wavelength.

The purpose of the present program is to develop a compact, integrated airborne system capable of mapping trends, gradients and anomalies in the distribution of surface water chlorophyll a present as planktonic algae.

## SECTION 2

## CONCLUSIONS

The laser-fluorosensor described in this report has been used to produce flight-line profiles of relative surface water chlorophyll a concentrations that vary over a range from 2 micrograms per liter (μg/l) to 20 μg/l at operating elevations from 200 meters (m) to 400 m. In its present form the laser fluorosensor is estimated to be capable of monitoring chlorophyll a concentrations down to 0.4 μg/l with a minimum signal to background noise ratio of 3. With the implementation of measures to increase the fluorescence signal and reduce the background noise, it will be possible to monitor chlorophyll a concentrations down to 0.1 μg/l with a minimum signal to background noise ratio of 20 under the same environmental conditions encountered in the present measurements.

Attempts to monitor changes in the concentration of blue-green algae by selectively exciting c-phycocyanin, a chlorophyll a-coupled photopigment unique to this freshwater algal division, were not successful. It is likely that the reason for this failure is the relatively low fluorescence cross section for this pigment in relation to that for chlorophyll a itself. It is possible that monitoring specific algal groups can be better accomplished by employing the multiwavelength excitation approach.

# SECTION 3

# RECOMMENDATIONS

The fully developed airborne laser fluorosensor will be able to produce contour maps of surface water chlorophyll a fluorescence. Due to the problems encountered in determining absolute values for the chlorophyll a fluorescence cross section and the diffuse water attenuation coefficients, it is not possible to produce a calibrated surface water chlorophyll a concentration map using airborne data alone. Rather this map must be calibrated in units of chlorophyll a concentration by the provision of ground truth chlorophyll a determinations obtained at one or two selected reference sites.

Presently, the degree of correlation between the airborne chlorophyll a fluorescence profiles and those for the corresponding ground truth chlorophyll a data is somewhat less than ideal, with linear correlation coefficients lying in the range between 0.77 and 0.95. Several reasons exist for this discrepancy between the airborne and ground truth data. Firstly, chlorophyll a determinations made according to presently accepted standards are both inefficient and of low reproducibility, whereas it is estimated that the airborne measurements, although prone to systematic errors, are reproducible to better than ± 5%. It is therefore suggested that investigations be conducted with the purpose of improving both the efficiency and accuracy of the method used for making chlorophyll a determinations at least to the point where the error is comparable to that for the airborne data. Secondly, the airborne chlorophyll a fluorescence signal is known to be dependent on changes in the surface water diffuse attenuation coefficients in addition to the chlorophyll a concentration. It is therefore proposed to obtain a relative indication of this variation by concurrently monitoring the water Raman emission signal. Theoretical and experimental considerations indicate that this approach is feasible. Modifications to the airborne laser-fluorosensor needed for measuring this Raman signal are straightforward and can be readily implemented with a minimum of modification to the present system. Finally, the dependence of the excitation cross section for chlorophyll a, $\sigma_C$, on the relative concentrations of the different algal color groups present in a given sample can be minimized by choosing a laser excitation wavelength at which the fluorescence cross section for the different algal color groups are more nearly equal. It is therefore proposed to conduct a series of airborne tests of the laser fluorosensor with the purpose of evaluating different excitation wavelengths for monitoring in vivo chlorophyll a.

A fully developed airborne laser-fluorosensor for making routine surveys of surface water chlorophyll a will require a number of additional features not available with the present system:

(1) Rapid installation in a variety of different aircraft will require a system considerably smaller than the present package which can be quickly and easily installed and either palletized or constructed of a number of discrete modular units suitable for rapid transportation and integration in the field.

(2) Provisions must be made for navigation data accurate to within a few meters in order that the airborne data can be reproduced in the form of a surface water map showing isopleths of constant chlorophyll a fluorescence. This can be accomplished by using an airborne range-positioning system which employs a microwave transmitter to interrogate two portable ground-based transponder units.

(3) A degree of real-time capability should be built into future laser fluorosensors for use as a quality control feature. A contour map of surface water chlorophyll a could then be produced and continuously updated on a cathode ray tube display for evaluation by the system operator. Current microprocessor technology is ideally suited to this application.

(4) Finally, the optical receiver for the laser fluorosensor will contain two extra spectral detector channels, in addition to those for the water Raman and chlorophyll a fluorescence signals. These additional detectors will monitor spectral intervals close to the water Raman and chlorophyll a fluorescence bands, thereby providing estimates of the background fluorescence due to dissolved organics at these two bands. These additional measurements are necessary whenever the concentration of dissolved organics is large or highly variable.

## SECTION 4

## REVIEW

Two passive remote-sensing methods are presently under development for monitoring distributions of surface water fluorescent tracer dyes and chlorophyll a.

In the first approach, photographic cameras or multichannel radiometric scanners are used to monitor the backscattered solar radiation in the visible and near infrared regions. These techniques generally provide imagery of high resolution with excellent spatial coverage, which can be used to obtain qualitative information on the location and extent of algae blooms, suspended sediment concentrations such as effluent plumes, oil spills, pollution-dumping sites and tracing dye dispersions. However, this approach has achieved only limited success in predicting the concentration of water quality parameters, in particular, chlorophyll a and suspended sediment. Much effort has been expended on formulating interpretation procedures for extracting information on chlorophyll a, e.g., references 1, 2 and 3, and suspended sediment content of surface waters, e.g., references 4, 5 and 6. With the aid of ground truth data, empirical interpretation schemes have been devised which are able to predict these parameters for waters containing particulates and algal distributions of a specific color, generally for a limited geographical region, but are seen to break down when applied to different water conditions. As yet, no universal model exists which can provide either absolute or relative chlorophyll a concentrations from passive radiometric data. These problems arise in part from the spectral overlap of the radiation backscattered by algae and particulate matter, in part from the differing nature of the spectral and scattering properties of different types of algae and suspended sediments, and in part from the presence of dissolved materials that impart a characteristic color to the water. In addition, the limited optical transmission of water, particularly in the near infrared, atmospheric backscatter, and surface water specular reflection of sky radiation further complicate the interpretation of the photographic or radiometric data. Finally, for successful operation of these devices, clear sky conditions in the absence of solar glitter are generally required. For passive airborne measurements made under cloud cover, signal-to-noise ratios are considerably reduced.

In the second approach, two airborne sensing techniques are worth mentioning with regard to their ability to remotely monitor surface water chlorophyll a. Both methods are passive but, rather than record changes in the backscattered solar spectrum, they detect variations in the solar-induced chlorophyll a fluorescence emission band at 685 nanometers (nm) that, ideally, can be directly related to the chlorophyll a concentration. Neville & Gower (7) used a multichannel spectrometer to monitor the ratio of the solar-induced fluorescence emission from the chlorophyll a band at 685 nm to the incident irradiance. Variations in airborne fluorescence measurements and the corresponding chlorophyll a ground truth data were found to be highly correlated.

The other passive fluorosensing approach presently under development employs a Fraunhofer Line Discriminator (FLD) to monitor the solar-induced target fluorescence observed in the region of the minimum of a solar Fraunhofer line (8). This device has been flown in a helicopter to map the surface water concentration of Rhodamine WT fluorescent tracing dye in the parts-per-billion (ppb) range (8) and more recently has been used to map the areal extent of marshland contaminated by an oil well blowout (9). When operated in the scanning mode, spatial coverage can be achieved by making a single pass over a target with navigation data provided by conveniently located ground-based transponder units. Surface water chlorophyll monitoring has not yet been attempted with this device, although it has been used to monitor the changes in chlorophyll a fluorescence of tree vegetation induced by geochemical soil anomalies (10). As with all passive remote-sensing devices operating in the optical region, it is dependent on bright daylight conditions for a usable signal-to-noise ratio. In addition, the specificity of the FLD technique is reduced both by the limited number and distribution of usable Fraunhofer lines and by the broad spectral nature of the solar source.

Recently, active remote monitoring systems employing lasers have been used to excite, detect and record the fluorescence of surface water targets. These airborne laser-fluorosensor systems operate by exciting fluorescence in a surface water volume, using a pulsed laser, and by collecting a small fraction of the multidirectional fluorescence emission, using a large-aperture telescope sighted on the excitation spot. The backscattered fluorescence pulse is then passed through a spectral analyzer onto a photodetector, and the resultant electronic signal is displayed, digitized and recorded. The schematic shown in Figure 1 illustrates the general optical principle involved in which the laser spot size might be on the order of 1 m to 2 m in diameter from a platform elevation of 200 m. Laser beams are highly directional, can have high average or peak power, and low beam divergence, and can be chosen or tuned to operate at any desired visible or ultraviolet (UV) excitation wavelength. Using laser excitation sources, acceptable signal-to-solar background-noise ratios can be obtained so that laser fluorosensors can be operated on a 24-hour basis. Three fluorescence parameters can be measured using this technique. Firstly, either the amplitude of the fluorescence signal, generally at the wavelength of peak emission, can be related to the concentration of a particular target of known fluorescence quantum efficiency, or, alternatively for an opaque or optically thick target, the quantum efficiency can be measured and used as an identifying feature. Secondly, the fluorescence emission spectra can be monitored and recorded at a number of discrete wavelengths. In addition, the fluorescence absorption or excitation spectrum can, in principle, be determined using a dynamically scanned tunable laser source, although such systems are not yet commercially available. Finally, the fluorescence decay time spectrum can be measured as a function of wavelength. All of these parameters are unique for a given fluorescence target and in combination constitute an unambiguous signature with which to

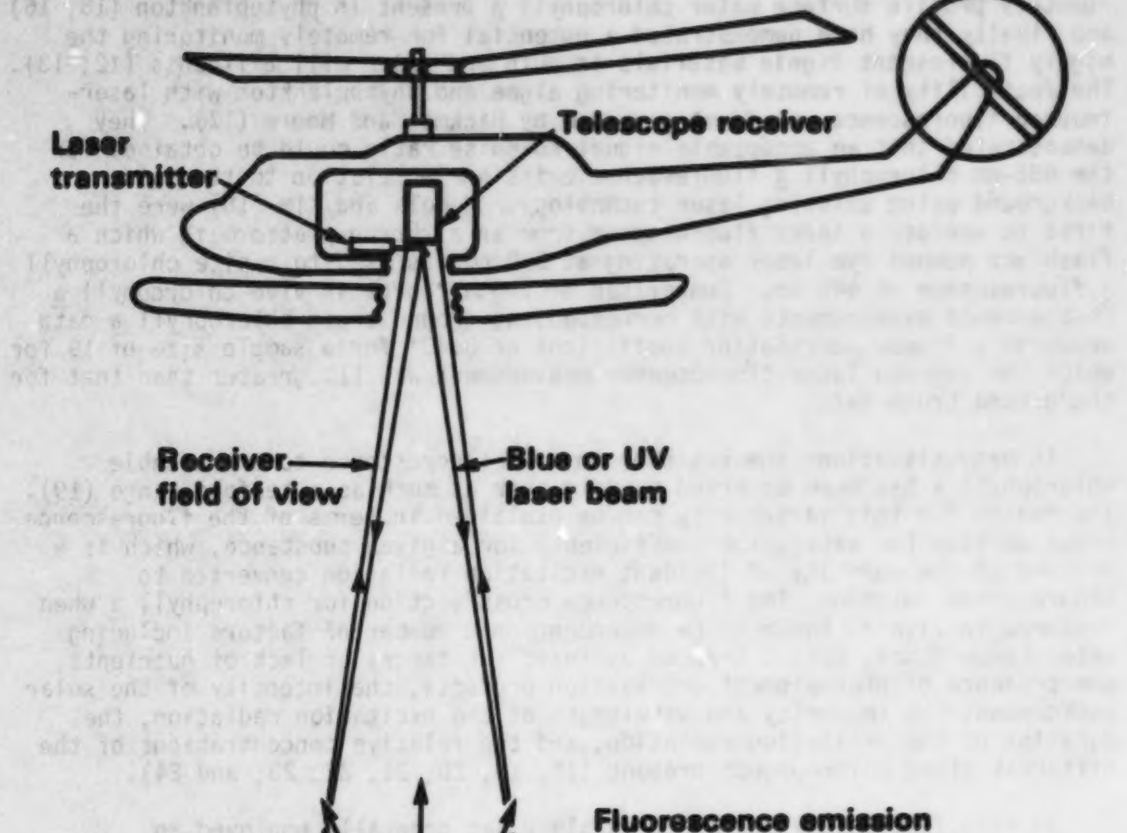
Telescope receiver
Laser transmitter
Receiver field of view
Blue or UV laser beam
Fluorescence emission from target
Surface water fluorescent target
Laser excited target area

Figure 1. Principle of operation of airborne laser fluorosensor.

identify or characterize the target in relation to the fluorescent background or to competing fluorescent targets.

Airborne laser fluorosensors have been used to detect surface water oil spills (11, 12, 13) and in one case to obtain the fluorescence emission spectra from a number of oil slicks (14). They have been successfully used to remotely profile surface water chlorophyll a present in phytoplankton (15, 16) and finally they have demonstrated a potential for remotely monitoring the highly fluorescent lignin materials in pulp and paper mill effluents (12, 13). The feasibility of remotely monitoring algae and phytoplankton with laser-induced fluorescence was first proposed by Hickman and Moore (17). They demonstrated that an acceptable signal-to-noise ratio could be obtained for the 685-nm chlorophyll a fluorescence emission in relation to the solar background using existing laser technology. Mumola and Kim (18) were the first to operate a laser fluorosensor from an airborne platform in which a flashlamp pumped dye laser operating at 590 nm was used to excite chlorophyll a fluorescence at 685 nm. Comparison of the airborne in vivo chlorophyll a fluorescence measurements with corresponding ground truth chlorophyll a data produced a linear correlation coefficient of 0.43* for a sample size of 19 for which the average laser-fluorosensor measurement was 11% greater than that for the ground truth data.

In many situations the ratio of in vivo fluorescence to extractable chlorophyll a has been observed to vary over as much as a tenfold range (19). The reason for this variability can be explained in terms of the fluorescence cross section (or excitation coefficient) for a given substance, which is a measure of the quantity of incident excitation radiation converted to fluorescence emission. The fluorescence cross section for chlorophyll a when measured in vivo is known to be dependent on a number of factors including water temperature, stress induced by toxic substances or lack of nutrients, the presence of photopigment degradation products, the intensity of the solar background, the intensity and wavelength of the excitation radiation, the duration of the excitation radiation, and the relative concentrations of the different algae color groups present (16, 19, 20, 21, 22, 23, and 24).

In vivo fluorometry performed on algae has generally employed an excitation wavelength of about 436 nm located close to the center of the blue-violet (Soret) absorption band of chlorophyll a. However, data presented by Friedman & Hickman (25) and by Mumola et al. (16) showed not only that the absolute value of the fluorescence cross section varies considerably between algal divisions or color groups but also that these cross sections exhibit considerable variability with wavelength. This problem arises because of the presence of different light-absorbing photosynthetic pigments among the different algal color groups. For example, blue-green algae or cyanophyta, often employed as indicators of eutrophic conditions in fresh water, contain, in addition to significant amounts of chlorophyll a and β-carotene, the blue pigment c-phycocyanin. This substance effectively blocks light absorption by the various chlorophyll and carotene pigments in the blue-green region and has a strong absorption band centered at 622 nm. This red radiation is then internally coupled directly into the chlorophyll a photosynthetic system. It therefore becomes clear that large changes in relative concentration of the different algal color groups on either a spatial or a temporal basis can be expected to have a significant effect on the accuracy of the predicted chlorophyll a concentration when only a single excitation wavelength is used. This will be true regardless of whether the in vivo measurements are made with a field fluorometer or an airborne laser-fluorosensor. Attempts to circumvent this problem have been made by employing an airborne laser-fluorosensor which sequentially operates at several excitation wavelengths (16,21). The number of laser wavelengths required is dictated by the number of algal color groups anticipated to be present in the surface water sample, with the wavelength of each laser tuned to lie close to the peak absorption wavelength for the given algal pigment. With a knowledge of the laser power, the water attenuation coefficients, and the fluorescence cross sections for each algal color group at each laser wavelength together with the fluorescence power levels at 685 nm excited by each laser, it is, in principle, possible to solve a series of laser fluorosensor equations to obtain the equivalent chlorophyll a concentration for each algal color group. On a test flight over the tidal flow region of the James River in Virginia, the 4-wavelength laser-fluorosensor was used to measure chlorophyll a concentrations for each of the golden, brown, green and blue-green algal color groups (16). Comparison of the total laser fluorosensor chlorophyll a values with corresponding ground truth produced a linear correlation coefficient of 0.90* for a sample size of 7. In this case the average laser-fluorosensor chlorophyll a value was approximately 28% below that for the ground truth data. A review of this multiwavelength approach by Browell (20) demonstrated that relatively small uncertainties in the values for the water attenuation coefficients and the algal color group fluorescence cross sections undergo considerable amplification when used to calculate the total in vivo chlorophyll a values. More recently, laboratory studies performed on known mixtures of four carefully controlled algal monocultures (each from a different color group) using the 4-wavelength laser fluorosensor, have obtained a linear correlation coefficient of 0.99 for covariation between the in vivo laser fluorosensor chlorophyll a measurements and the corresponding extractable chlorophyll a data (21). However, the stable laboratory conditions employed for these measurements are neither typical nor representative of the true field environment. A number of unpredictable factors not present in these measurements are known to have a significant influence on the algal color group fluorescence cross sections. These are stress induced by nutrient limitations, the effect of toxic substances, changes in water temperature, age of the algal communities, the intensity of the solar background radiation and the wavelength, intensity and duration of the fluorescence excitation light source (19, 20, 22). In the absence of either in situ or remotely sensed data relating to the water attenuation coefficient and algal fluorescence cross sections, Browell (20) has suggested that both single and multi-wavelength laser fluorosensors are, at best, capable of providing only qualitative or relative measure of surface water chlorophyll a concentration.

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*The linear correlation coefficient was calculated by the present authors from graphical data presented by Mumola & Kim (18).

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*The linear correlation coefficient was calculated by present authors from graphical data presented by Mumola et al. (16).

In light of these observations and with a view to reducing system complexity, a program has been initiated to design, build and test a single excitation wavelength airborne laser-fluorosensor. The remotely sensed fluorescence signature will then be used to produce surface water maps showing isopleths of surface water chlorophyll a concentration as illustrated in Figure 2. Whereas field fluorometers almost universally employ a wavelength of about 440 nm to excite fluorescence in planktonic algae (24), other studies, based on laboratory investigations performed on water samples and algal monocultures suggest that wavelengths in the 600-nm region are more suitable for monitoring the overall chlorophyll a level for disparate mixtures of algae (15, 16, 25). In particular, from the measurements made on specific algal color groups presented in Reference 16, it is apparent that at 620 nm the fluorescence excitation cross sections for the green, blue-green, red and golden brown algal color groups are all approximately equal. With these observations in mind, a flexible approach was adopted allowing different excitation wavelengths to be evaluated with a view to optimizing both the degree of correlation attainable between the airborne and ground truth data and also system sensitivity. In particular it was planned to employ a wavelength of 440 nm to facilitate comparison between the airborne data and the fluorometrically determined ground truth data and also a wavelength of 620 nm for the reasons stated above.

The principal advantage of the fluorometric approach, whether active or passive, over the reflectance spectroscopy approach is its provision of raw data directly proportional to chlorophyll a concentration. The data can then be analyzed without the need for empirical interpretation models established beforehand with ground truth data. In this respect the airborne laser-fluorosensor is similar in principle and operation to field fluorometers that are widely used for in situ monitoring of both surface and subsurface in vivo chlorophyll a (19, 24, 26). The principal difference between these two fluorometric techniques is that the in situ system employs a constant pathlength cell, whereas, with the remote sensing approach, the effective sample pathlength and resultant fluorescence signal are dependent on the variable optical attenuation lengths for the laser and fluorescence wavelengths. A method for compensating for this variable sample length by concurrently measuring the water Raman band emission is discussed in Section 8.

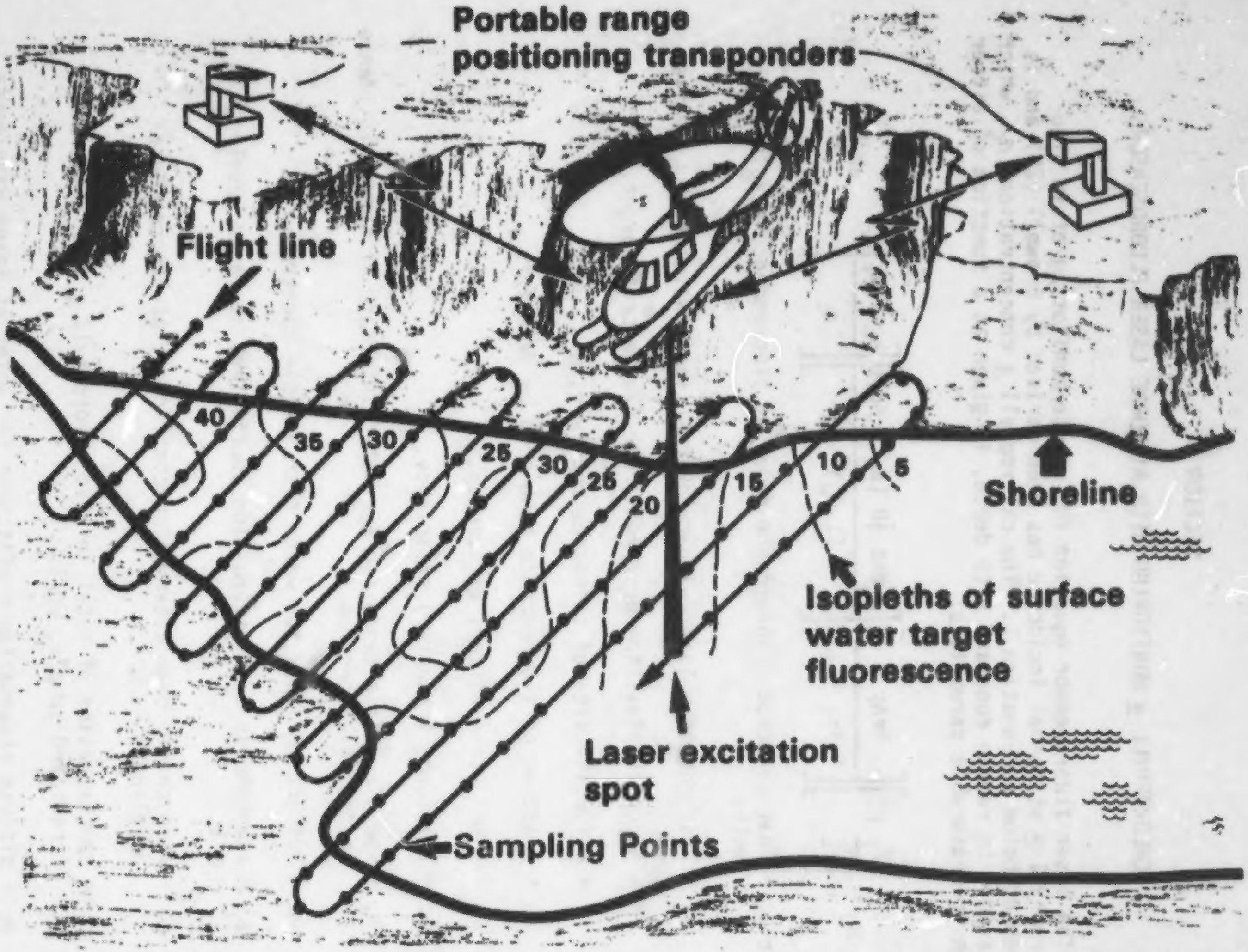
Portable range
positioning transponders
Flight line
40
35
30
25
30
25
20
15
10
5
Shoreline
Isopleths of surface
water target
fluorescence
Laser excitation
spot
Sampling Points

Figure 2. Schematic illustrating possible mode of operation of an airborne laser fluorosensor for mapping surface water chlorophyll a distributions.

## SECTION 5

## CHLOROPHYLL a MONITORING WITH AIRBORNE LASER FLUOROSENSORS

The laser fluorosensor equation for remote monitoring of in vivo chlorophyll a at normal incidence has been derived by Browell (20) and is presented below as Equation 1. The chlorophyll a concentration, $n_c$, which is assumed to remain constant with depth, is given as a function of either known or measurable parameters:

$$n_c = \left[\frac{P_F}{P_L} H^2\right]\left[\frac{4\pi\Delta_F}{T\ \eta_{Tr}\ \eta_{Rec}}\right]\left[\frac{\mu_w^2 \exp\{H(\beta_L + \beta_F)\}}{(1-R_w)^2}\right]\left[\frac{k_L + k_F}{\sigma_c}\right] \quad \mu g/l \qquad (1)$$

where $P_F$ = Peak detected fluorescence power at fluorescence wavelength F, watts.

$P_L$ = Peak detected laser output power at laser wavelength L, watts.

$H$ = Aircraft elevation or range above fluorescent target, m.

$T$ = Effective area of telescope receiver, $m^2$.

$\Delta_F$ = Fraction of fluorescence band seen by detector.

$\eta_{Tr}$ = Laser (transmitter) efficiency.

$\eta_{Rec}$ = Telescope (receiver) efficiency.

$R_w$ = Specular reflectance for air-water interface at normal incidence for visible spectrum.

$\mu_w$ = Refractive index for water over visible spectrum.

$\beta_L$ = atmospheric beam attenuation coefficient at laser wavelength $m^{-1}$.

$\beta_F$ = atmospheric beam attenuation coefficient at fluorescence wavelength F, $m^{-1}$.

$n_c$ = concentration of total in vivo chlorophyll a from algae in irradiated sample, µg/l.

$k_L$ = diffuse attenuation coefficient for water at laser wavelength L, $m^{-1}$.

$k_F$ = diffuse attenuation coefficient for water at fluorescence wavelength F, $m^{-1}$.

$\sigma_C$ = in vivo cross section for chlorophyll a fluorescence over complete fluorescence band centered at 685 nm when excited at laser wavelength L, $m^2/mg$.

Factors within the first set of square brackets in Equation 1 are measured directly from each digital or analog laser fluorosensor record obtained for a given sampling station. Those within the second set of square brackets are known or measurable laser fluorosensor system constants, whereas those within the third set of brackets relate to known environmental factors. However, it is the difficulty in anticipating the behavior of the environmental factors within the fourth set of square brackets, specifically $\sigma_C$, $k_L$ and $k_F$ that constitutes the major limitation to the implementation of this remote-sensing technique. The assumptions made during the derivation of Equation 1, in particular those regarding the parameters $\sigma_C$, $k_F$ and $k_L$, are discussed below:

(1) It is assumed that the combined reflected and backscattered sky and backscattered solar background levels have been subtracted from the detector signal to obtain $P_F$. In addition, it is assumed that the laser induced background signal at 685 nm due to dissolved organics is negligible in relation to the chlorophyll a fluorescence signal.

(2) The surface water excitation volume is assumed to lie completely within the telescope field of view.

(3) By integrating the fluorescence signal to infinite depth it is necessary to assume that the total water depth is at least an order of magnitude greater than the characteristic attenuation lengths given by $(k_F)^{-1}$ and $(k_L)^{-1}$. It should be noted that light penetration is characterized by the diffuse attenuation coefficient k rather than by the related beam attenuation coefficient $\alpha$, as both singly and multiply scattered excitation and fluorescence radiation contribute to the measured fluorescence signal (20, 27). In addition, values for k are generally smaller than those for $\alpha$ at a given wavelength because multiple scattering effects result in an increased level of illumination at a given depth, whereas $\alpha$, which is measured with a narrow collimated beam, accounts for only single scattering losses. Specifically, k accounts for absorption and backward-scattering losses whereas $\alpha$ accounts for absorption and both forward and backward-scattering losses. Further discussion of the relationship between k and $\alpha$ is provided in Section 7 (e).

(4) Inherent in the integration of the fluorescence signal to infinite depth is the assumption that $n_C$, $k_L$, $k_F$ and $\sigma_C$, all remain constant with depth. It is generally considered that, if $k_L$ and $k_F$ remain constant to depths at least double those defined by the attenuation lengths $(k_L)^{-1}$ and $(k_F)^{-1}$, then errors induced in $n_C$ as calculated from Equation 1, can be kept below 10%. Concurrent vertical profiles of the beam attenuation coefficient $\alpha$ and the in vivo chlorophyll a fluorescence obtained in a marine

environment by Kiefer and Austin (28) have shown that the transmissometer and the fluorometer signals remain constant down to 10 meters with $\alpha \simeq 1\ m^{-1}$. Similar measurements made in a fresh-water environment by Baker and Baker (29) have shown that both $n_C$ and $\alpha$ remained constant down to about 3 meters for $\alpha > 4m^{-1}$. Although no infallible rule exists which guarantees that $n_C$, $\sigma_C$, $k_L$ and $k_F$ will remain constant to depths characterized by $2(k^{-1})$ or $2(\alpha^{-1})$, most coastal, estuarine and inland waters have $\alpha$ values greater than $1m^{-1}$, such that wind-induced mixing should ensure that this surface layer will remain homogeneous with regard to both particulate and algal matter.

(5) The assumption is made that the fluorescence lifetime for in vivo chlorophyll a is less than or equal to the laser pulse width. In the present case, with a laser pulse width of about 200 nsec, and a chlorophyll a fluorescence lifetime of less than 1 nanosecond (nsec) (30), this condition is clearly met. However, in situations where the laser pulse width is less than or equal to the fluorescence lifetime, the value of $P_F$ would be reduced in relation to $P_L$ due to the effect of pulse spreading induced by the fluorescence decay phenomenon. This problem can be avoided by either applying a suitable correction to the peak power measurements or measuring pulse energy rather than peak power for both the laser and fluorescence signals.

(6) For clear atmospheric conditions over limited ranges of the order of 300 m, the atmospheric attenuation term exp $\{H(\beta_L + \beta_F)\}$ remains essentially constant with a value close to unity, so that the effect of the terms within the third set of square brackets can be considered to be constant.

(7) Several assumptions are made with regard to the fluorescence emission cross section $\sigma_C$. Firstly, it is assumed to remain constant with both depth and horizontal location. Secondly, since $\sigma_C$ is defined purely in terms of chlorophyll a it is assumed that the fluorescence emission at 685 nm is due solely to the excitation of chlorophyll a pigment. Finally, it is assumed that the percentage proportions of the different algal divisions (color groups) remain constant with depth and horizontal location. It is necessary to make this latter assumption because, as mentioned in Section 4, the magnitude of the cross section for in vivo chlorophyll a fluorescence varies considerably between algal divisions.

(8) Similarly, the diffuse water attenuation coefficients $k_L$ and $k_F$ are assumed to remain constant with horizontal surface location and also to be independent of the chlorophyll a concentration $n_C$.

To obtain a precise value of $n_C$ by use of Equation 1, estimates of $\sigma_C$, $k_L$ and $k_F$ must be obtained, generally, by making in situ measurements at a reference sampling station in the water surface being surveyed, on the assumption that these reference values are representative of all possible sampling points in the water surface. However, the laboratory technique for estimating in vivo values for $\sigma_C$ for a given sample is not easily implemented, requiring specialized equipment and trained personnel. Rather, this measurement should be made with an in situ fluorometer so that the various environmental factors mentioned in Section 4, which are known to influence $\sigma_C$, remain unchanged. With a view to avoiding this requirement for making in situ measurements, the present airborne experiments are therefore aimed at obtaining a relative measure of surface water chlorophyll a concentration based on the assumption that $n_C$ is directly proportional to the measured airborne data represented by the factor $(P_F/P_L)H^2$, which is the term in the first set of square brackets in Equation 1. All other parameters, although unknown, are assumed to remain constant, including $\sigma_C$, $k_L$ and $k_F$. The success of this technique for monitoring surface water chlorophyll a will then be gauged by rating the degree of covariation obtained between values of $(P_F/P_L)H^2$ and the extracted chlorophyll a determinations made on corresponding surface water ground truth samples.

# SECTION 6

# INSTRUMENTATION

The laser fluorosensor consists of a laser transmitter and telescope receiver, which are mounted in a lightweight aluminum box in a non-coaxial configuration, as shown schematically in Figure 3. Airborne operations are conducted from a Bell UH/1D H (Huey) helicopter, with the system mounted in a central location over a clear hole cut between two stringers just forward of the transmission housing.

The system operates in a fixed downward-looking mode which at typical aircraft ground speeds of 20 m/sec and a laser repetition rate of 1 pulse per second (pps), gives a ground footprint spacing of 20 m. At an above target elevation of 200 m, the laser beam divergence of 6 mrad produces a water surface spot size of 1.2 m diameter. As the ground resolution of the system is essentially set by the (20-m) spacing between laser excitation spots, it would be advantageous to utilize a laser spot diameter similar to this interspot spacing to smooth out the effects of small-scale fluctuations in the concentration of surface water algae. This advantage can be gained by increasing the aircraft height H without increasing the laser beam divergence or the telescope field of view. A limitation to this expedient is set by the $1/H^2$ drop-off in the fluorescence signal with increasing H. As a result, the sky and solar background signal and noise within the telescope field of view eventually compete with and ultimately dominate the fluorescence signal. In this case the signal-to-background noise-ratio also varies as $1/H^2$. Consequently, doubling the aircraft height produces a fourfold reduction in the signal-to-background noise ratio (31). Another approach to increasing the laser excitation spot size would be to double the laser beam divergence $\theta$ together with the telescope field of view at a given aircraft altitude. In this case the signal-to-background noise varies as $1/\theta$, so that doubling the system field of view halves the signal-to-background noise-ratio (31). However, this approach is much more difficult to implement than that achieved by changing the aircraft height due to the problems involved in continuously and simultaneously adjusting the fields of view for both laser and telescope. A better approach would be to increase the laser repetition rate, thereby increasing the sampling frequency so that the effects of algal patchiness can, if desired, be smoothed out by low pass filtering the measured chlorophyll a profile.

Adjustment of the position of the surface water laser spot is achieved with the laser beam steering mirror shown in Figure 3, which positions the laser spot at the center of the telescope field of view. This micrometer adjustment is made during each flight at a prescribed altitude by maximizing the amplitude of the detected surface water chlorophyll a fluorescence signal at a given location.

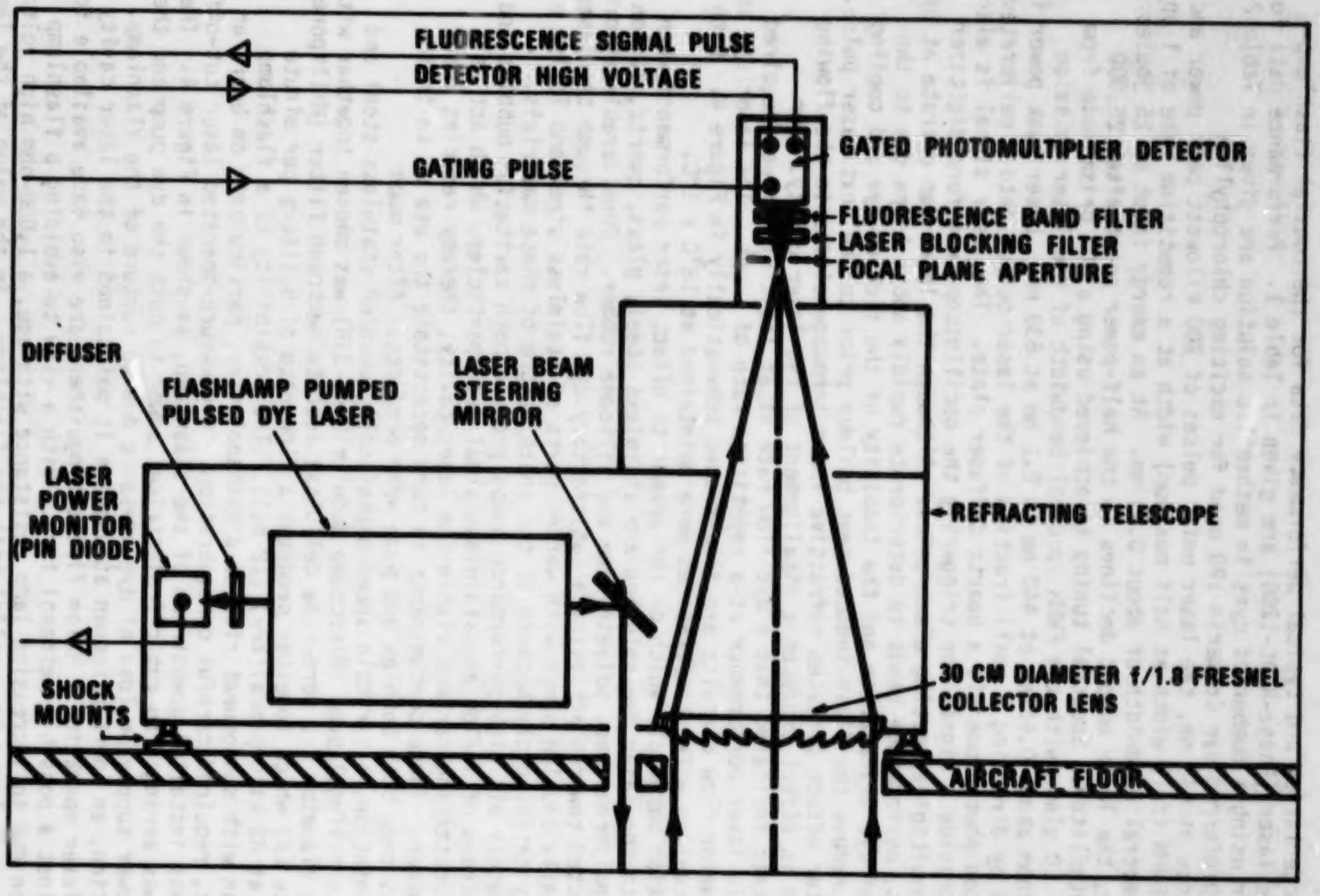
FLUORESCENCE SIGNAL PULSE
DETECTOR HIGH VOLTAGE
GATING PULSE
GATED PHOTOMULTIPLIER DETECTOR
FLUORESCENCE BAND FILTER
LASER BLOCKING FILTER
FOCAL PLANE APERTURE
DIFFUSER
FLASHLAMP PUMPED PULSED DYE LASER
LASER BEAM STEERING MIRROR
REFRACTING TELESCOPE
LASER POWER MONITOR (PIN DIODE)
30 CM DIAMETER f/1.8 FRESNEL COLLECTOR LENS
SHOCK MOUNTS
AIRCRAFT FLOOR

Figure 3. Optical diagram of airborne laser fluorosensor for monitoring surface water fluorescence signal.

## LASER TRANSMITTER SYSTEM

Characteristics and typical performance data for the coaxial flashlamp-pumped dye laser (Phase-R DL-1200) are given in Table 1. Performance data for this laser using a number of dyes in methanolic solution are given in Table 2. With the preferred dye Coumarin 120 used for exciting chlorophyll a fluorescence at 440 nm, the laser emits pulses of 200 kilowatt peak power and 200 nsec FWHM (full width at half maximum) width at a repetition rate of 1 pps with a spectral bandwidth of about 0.45 nm. At an energy input of 25 joules per pulse, the laser output declines to the half-power point after 25,000 joule shots/liter. Spectral tuning is achieved using a 60° prism made from Schott SF 10 glass with the FWHM spectral bandwidth of the laser emission varying from about 0.45 nm at 440 nm to 2.1 nm at 639 nm. Laser peak power is monitored by directing a small fraction of the laser output onto a calibrated PIN silicon photodiode via a quartz diffuser plate. The laser signal is also used to provide a signal for triggering the oscilloscope waveform digitizer and photomultiplier gating electronics. Although the laser can operate at up to 10 pps, performance tends to deteriorate rapidly above 1 pps due to the effects of dye degradation and the inability of the flowing dye and cooling water to remove the flash-induced heat buildup prior to the next laser pulse. This latter effect produces refractive index inhomogeneities in the flowing dye which in effect produces a misalignment of the laser cavity. Our measurements indicated that a dye flow rate of at least 5l/min was required for stable laser performance at a repetition rate of 1 pps. The laser dye and cooling water flow circuits are illustrated schematically in Figure 4. Both dye and cooling water temperatures were maintained at 18°C ± 1°C. Construction materials which do not appear to effect laser performance when exposed to the laser dye solution are stainless steel, glass, quartz, Teflon, Delrin, polypropylene, polyethylene and silicone rubber. Considerable effort was directed towards achieving a satisfactory dye flow rate through the laser. Magnetically coupled pumps with wetted parts of stainless steel and Teflon are generally satisfactory because of the inert nature of these materials. Unfortunately all high-performance pumps produce both cavitation bubbles and a steady stream of Teflon and stainless steel wear particles, which act as optical scattering centers within the laser cavity, thereby reducing laser output power. These contaminants in turn necessitate the use of an inline filter to trap both bubbles and pump wear products. After much experimentation, a variable speed magnetically coupled stainless steel and Teflon centrifugal pump (Micropump Model #10-41-316) was chosen together with a 142-mm diameter 1-$\mu$ pore-size cellulose acetate membrane filter (Millipore Cellotate EA) which together produced a flow rate of 5 liters per minute (l/min) at 83 kilo pascal(kPa) (12 psi). The possibility of a flashlamp explosion with subsequent risk of a methanol fire, particularly on board an aircraft, required careful consideration. A pressure-operated laser cut-off switch was installed downstream of the flashlamp, as shown in Figure 4. The switch was arranged to cut off electrical power to both the dye pump and the laser power supply on loss of dye pressure due to rupture of the flashlamp. In addition, an inert nitrogen atmosphere is maintained in the laser cavity during laser operation. Halon fire extinguishers are also made available for use against a possible methanol fire. With a view to avoiding a flashlamp explosion due to increasing lamp resistance with age, a 1,000-ohm high voltage resistor was placed in parallel with the flashlamp. As the value of the lamp

TABLE 1. AIRBORNE LASER-FLUOROSENSOR CHARACTERISTICS

|  |  |
|---|---|
| **LASER TRANSMITTER** |  |
| Peak power | 100 KW - 300 KW |
| Pulse width (FWHM) | 200 nsec - 280 nsec |
| Pulse energy | 40 mj - 70 mj |
| Beam divergence | 6 mrad |
| Spectral bandwidth | 0.45 nm - 2.1 nm |
| Repetition rate | 0.1 pps - 10 pps |
| Degree of polarization | Linear to within 1 part per 100 |
| **TELESCOPE RECEIVER** |  |
| Refractor | 30 cm diam., f/1.8, Acrylic Fresnel |
| Focal plane aperture | 4 mm diam. |
| Chlorophyll a fluorescence filter | 685 nm, 13 nm FWHM,<br>64% Transmission |
| Laser blocking filter | Corning 2-64 |

TABLE 2. LASER DYES EVALUATED FOR USE IN COAXIAL FLASHLAMP-PUMPED DYE LASER EMPLOYED IN AIRBORNE LASER FLUOROSENSOR

| Laser dye[A] | Molecular Weight | Molecular Concentration (M)[B] | Peak Wavelength (nm) | Tuning Range (nm) | Peak Power (KW)[C] | Spectral Bandwidth FWHM (nm) | Pulsewidth FWHM (nsec) | Dye Lifetime Joule-Shots/l |
|---|---|---|---|---|---|---|---|---|
| Coumarin 120 (EOC) | 175.19 | $5 \times 10^{-4}$ | 452 | 432-468 | 200 | 0.45 | 200 | 25,000 |
| Coumarin 339 (EOC) | 215.25 | $2 \times 10^{-4}$ | 482 | 440-492 | 210 | 0.93 | 240 | 20,000 |
| Coumarin 311 (EOC) | 203.24 | $2 \times 10^{-4}$ | 485 | 442-492 | 255 | 0.80 | 225 | 50,000 |
| Coumarin 314 (EOC) | 313.35 | $1 \times 10^{-4}$ | 520 | 485-541 | 300 | 1.25 | 250 | 50,000+ |
| Coumarin 522F (EXC) | 283.25 | $2 \times 10^{-4}$ | 534 | 502-572 | 240 | 0.97 | 250 | 50,000+ |
| Rhodamine 110 (EOC) | 366.80 | $5 \times 10^{-5}$ | 570 | 545-590 | 220 | 2.00 | 280 | 100,000+ |
| RhodamineB Perchlorate (EOC) | 543.02 | $5 \times 10^{-5}$ | 639 | 595-655 | 270 | 2.10 | 275 | 250,000 |

A) EOC: Eastman Organic Chemicals, Rochester, NY; EXC: Exciton Chemical Company, Dayton, OH.

B) Solvent: Spectral grade methanol.

C) Phase-R DL 1200 Laser: Input energy-25 Joules; Dye temperature-65°F; Dye flow rate- 5 l/min; Repetition rate-1 pulse/sec.

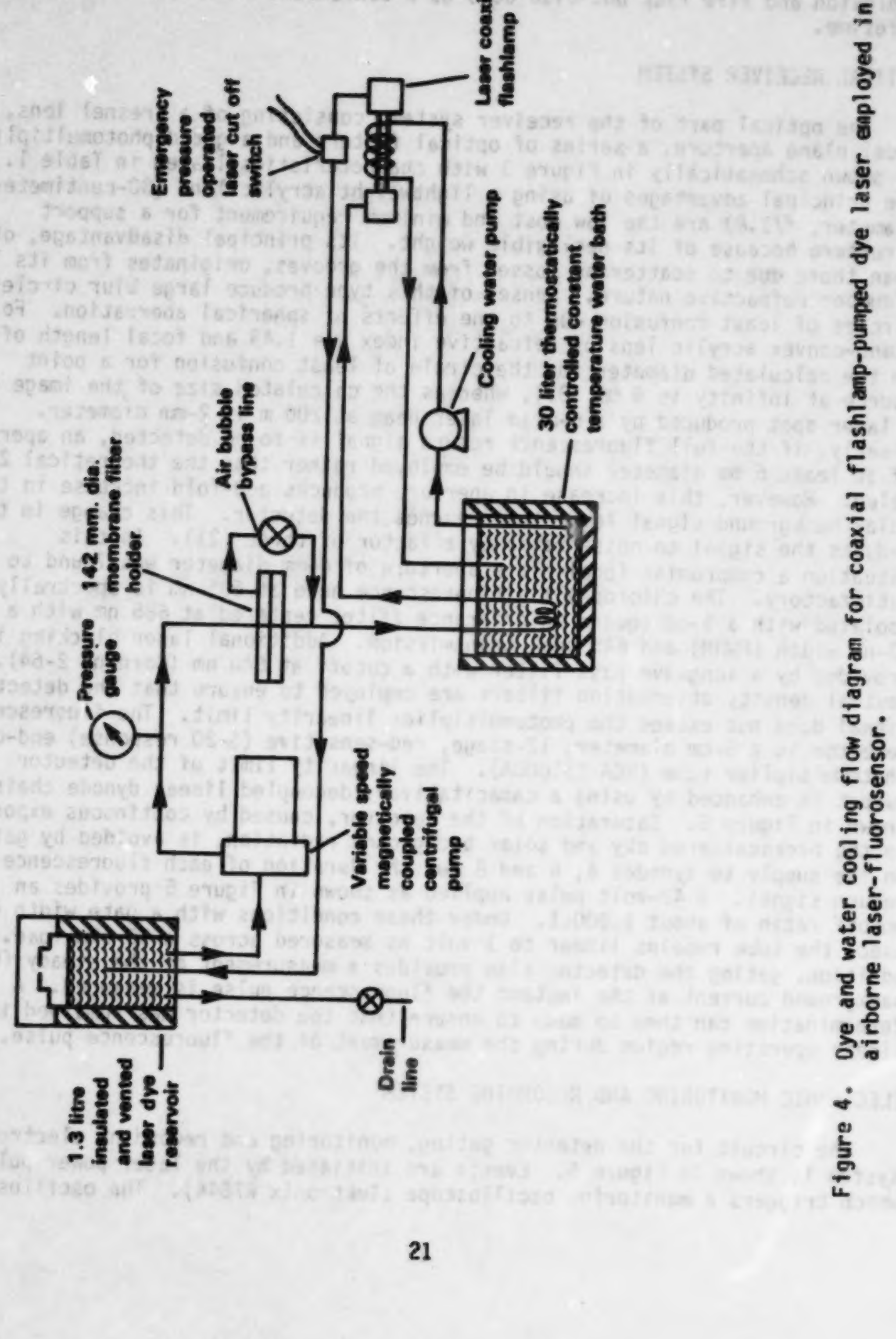
Laser coaxial flashlamp
Emergency pressure operated laser cut off switch
Cooling water pump
30 liter thermostatically controlled constant temperature water bath
Air bubble bypass line
142 mm. dia. membrane filter holder
Pressure gauge
Variable speed magnetically coupled centrifugal pump
1.3 litre insulated and vented laser dye reservoir
Drain line

Figure 4. Dye and water cooling flow diagram for coaxial flashlamp-pumped dye laser employed in airborne laser-fluorosensor.

resistance increases to that of the resistor, a point is reached at which increased current flow through the resistor will cause the resistor to fail in preference to the lamp (32). This expedient not only avoids a flashlamp explosion and fire risk but also acts as a convenient indicator of flashlamp lifetime.

## OPTICAL RECEIVER SYSTEM

The optical part of the receiver system, consisting of a Fresnel lens, a focal plane aperture, a series of optical filters and a gated photomultiplier, is shown schematically in Figure 3 with characteristics listed in Table 1. The principal advantages of using a lightweight acrylic lens (30-centimeter diameter, f/1.8) are the low cost and minimum requirement for a support structure because of its negligible weight. Its principal disadvantage, other than those due to scattering losses from the grooves, originates from its low f/number refractive nature. Lenses of this type produce large blur circles or circles of least confusion due to the effects of spherical aberration. For a plano-convex acrylic lens of refractive index $\mu = 1.49$ and focal length of 30 cm the calculated diameter for the circle of least confusion for a point source at infinity is 6 mm (33), whereas the calculated size of the image of a laser spot produced by a 6-mrad laser beam at 200 m is 2-mm diameter. Clearly, if the full fluorescence return signal is to be detected, an aperture of at least 6 mm diameter should be employed rather than the theoretical 2 mm value. However, this increase in aperture produces a 9-fold increase in the solar background signal level that reaches the detector. This change in turn reduces the signal to noise ratio by a factor of three (31). In this situation a compromise focal plane aperture of 4-mm diameter was found to be satisfactory. The chlorophyll a fluorescence band at 685 nm is spectrally isolated with a 5-cm square interference filter centered at 685 nm with a 13-nm width (FWHM) and 64% peak transmission. Additional laser blocking is provided by a longwave pass filter with a cutoff at 640 nm (Corning 2-64). Neutral density attenuation filters are employed to ensure that the detected signal does not exceed the photomultiplier linearity limit. The fluorescence detector is a 5-cm diameter, 12-stage, red-sensitive (S-20 response) end-on photomultiplier tube (RCA C31000A). The linearity limit of the detector output is enhanced by using a capacitatively decoupled linear dynode chain as shown in Figure 5. Saturation of the detector, caused by continuous exposure to the backscattered sky and solar background radiation, is avoided by gating on the supply to dynodes 4, 6 and 8 for the duration of each fluorescence return signal. A 42-volt pulse applied as shown in Figure 5 provides an on/off ratio of about 1,000:1. Under these conditions with a gate width of 3 μsec, the tube remains linear to 1 volt as measured across a 50-ohm load. In addition, gating the detector also provides a measurement of the steady (DC) background current at the instant the fluorescence pulse is measured. A determination can then be made to ensure that the detector has remained in the linear operating region during the measurement of the fluorescence pulse.

## ELECTRONIC MONITORING AND RECORDING SYSTEM

The circuit for the detector gating, monitoring and recording electronic system is shown in Figure 6. Events are initiated by the laser power pulse, which triggers a monitoring oscilloscope (Tektronix R7844). The oscilloscope

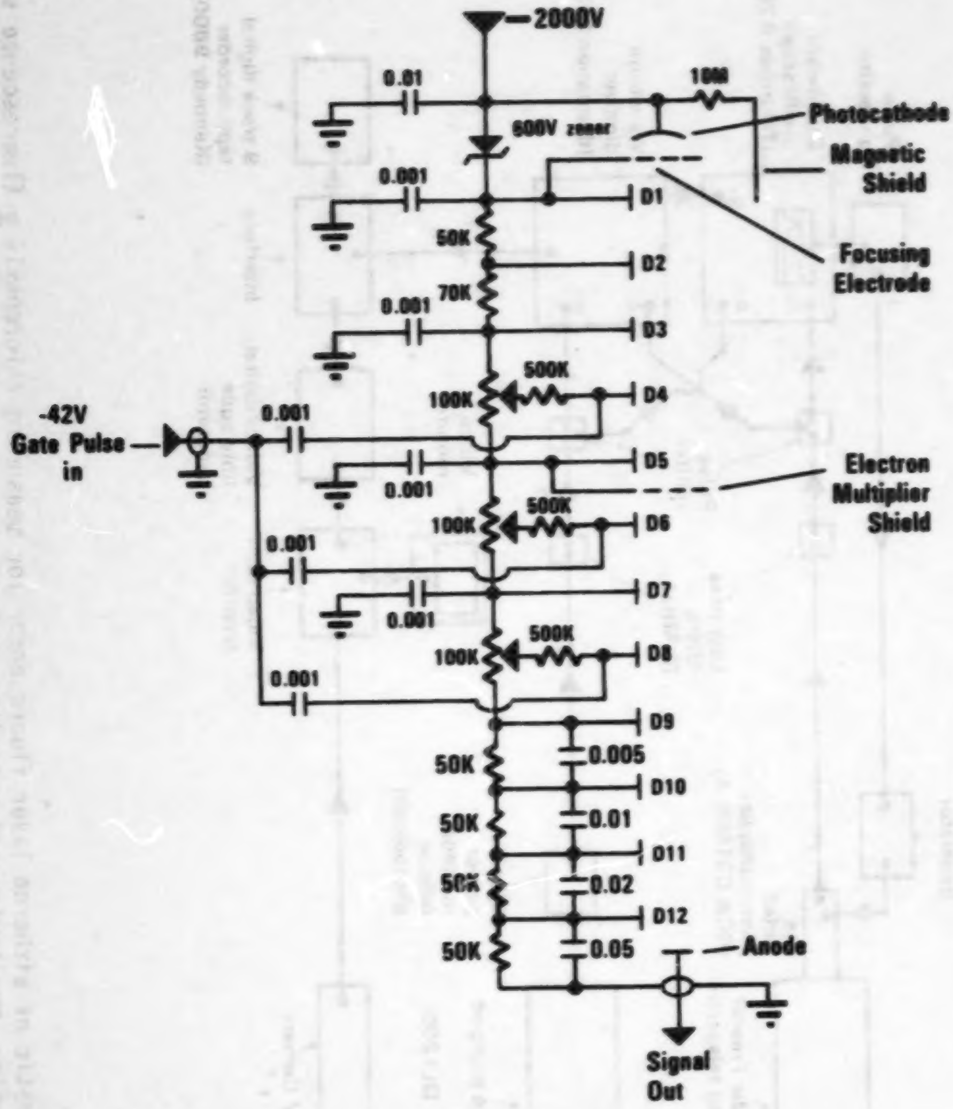
-2000V
0.01
10M
600V zener
Photocathode
Magnetic Shield
0.001
D1
50K
D2
70K
Focusing Electrode
0.001
D3
500K
100K
D4
-42V Gate Pulse in
0.001
D5
0.001
Electron Multiplier Shield
500K
100K
D6
0.001
D7
0.001
500K
100K
D8
0.001
D9
50K
0.005
D10
50K
0.01
D11
50K
0.02
D12
50K
0.05
Anode
Signal Out

Figure 5. Gate and voltage divider circuit diagram for RCA C31000A photomultiplier.

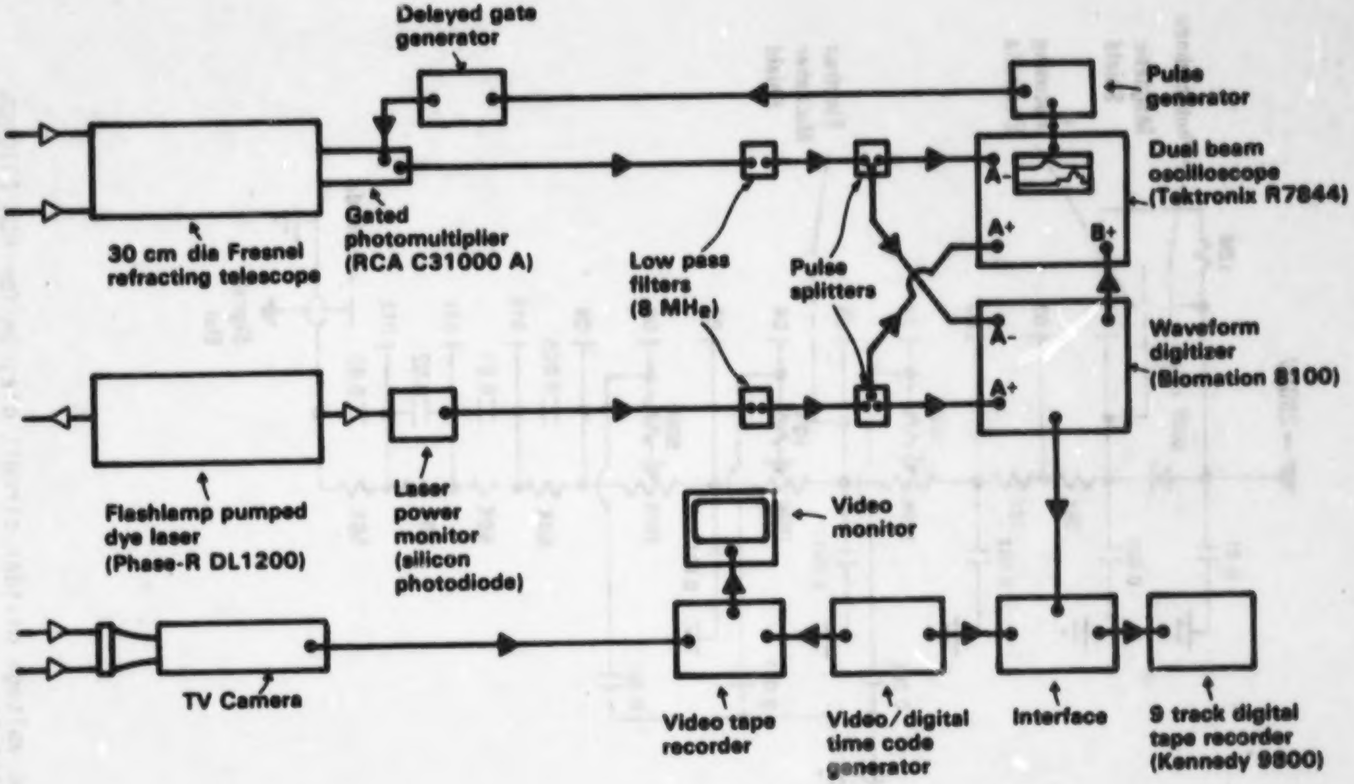
Delayed gate generator
Pulse generator
Dual beam oscilloscope (Tektronix R7844)
A-
A+
B+
30 cm dia Fresnel refracting telescope
Gated photomultiplier (RCA C31000 A)
Low pass filters (8 MHz)
Pulse splitters
Waveform digitizer (Biomation 8100)
A-
A+
Flashlamp pumped dye laser (Phase-R DL1200)
Laser power monitor (silicon photodiode)
Video monitor
TV Camera
Video tape recorder
Video/digital time code generator
Interface
9 track digital tape recorder (Kennedy 9800)

Figure 6. Schematic of airborne laser fluorosensor for measuring chlorophyll a fluorescence showing detection, monitoring and recording systems.

time base gate output is then used to trigger a pulse generator which in turn initiates formation of a delayed detector gate pulse. The delay period for the detector gate pulse is adjusted so that the detector is activated several hundred nanoseconds before the arrival of the backscattered fluorescence signal. This series of events is conveniently illustrated by the oscillogram shown in Figure 7. In this case, for a height of 309 m (1,103 ft), the detector was gated on 1.1 μsec after lasing. Also shown are the laser power pulse and the chlorophyll a fluorescence return from the algae, which is superimposed on the steady 3-μsec background signal. This background signal, consisting of both sky and solar backscatter and sky surface specular reflection components, does not constitute a serious noise source for the present laser fluorosensor system. However, the same cannot be said of the effects of solar glitter, viz., the direct specular surface reflection of the solar disc seen by the detector. In this case, the solar glitter background signal not only contributes significantly to the overall signal noise level but often drives the detector into a region of non-linearity or saturation. In the light of these observations it was found advisable to avoid making flights over rough surface water at times of high sun angle when solar glitter can be seen by a downward-looking sensor. The most favorable time was generally in the early morning hours, when the sun angle is low and the water surface is calm. The overall signal waveform such as that shown in Figure 7 is also digitized into 512 channels, each 10 nsec wide with 8-bit resolution over a 5.12-μsec period using a fast waveform digitizer (Biomation 8100). This digital data is then dumped from an internal buffer memory through an interface bus onto magnetic tape using a 9-track digital tape recorder (Kennedy 9800). The effective bandwidth of both digital and analog electronics is approximately 8 MHz dictated principally by the 50-ohm low-pass filters used to reduce the amplitude of photon and electromagnetic interference (EMI) noise. These filters are conveniently made from lossy 50-ohm cable whose low pass cutoff frequency is dependent on the cable length*. These filters are relatively cheap and easy to install with suitable (BNC) connectors and transmit signals free of ringing or line reflections.

Navigation, fluorescence target evaluation and track recovery were facilitated using a TV camera with a wide angle lens mounted directly to the laser fluorosensor module and boresighted with the laser beam. The helicopter flight path over a chosen water surface target can then be viewed directly on a video monitor and the same video image recorded on a video cassette. In order to use this video recording to assist with interpretation of the laser fluorosensor data, a video digital time-code generator was used to index each digital laser fluorosensor record and each video frame with a time signal to the nearest 1/100 second. An approximate ground location for each laser fluorosensor record can then be established.

Despite considerable effort expended on the digital recording equipment, satisfactory operation of this part of the system was not achieved for several reasons. The high ambient daytime temperatures encountered in the southern Nevada area often produce temperatures inside the helicopter in excess of

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*Manufactured by CAPCON, Inc. NY, NY 10001.

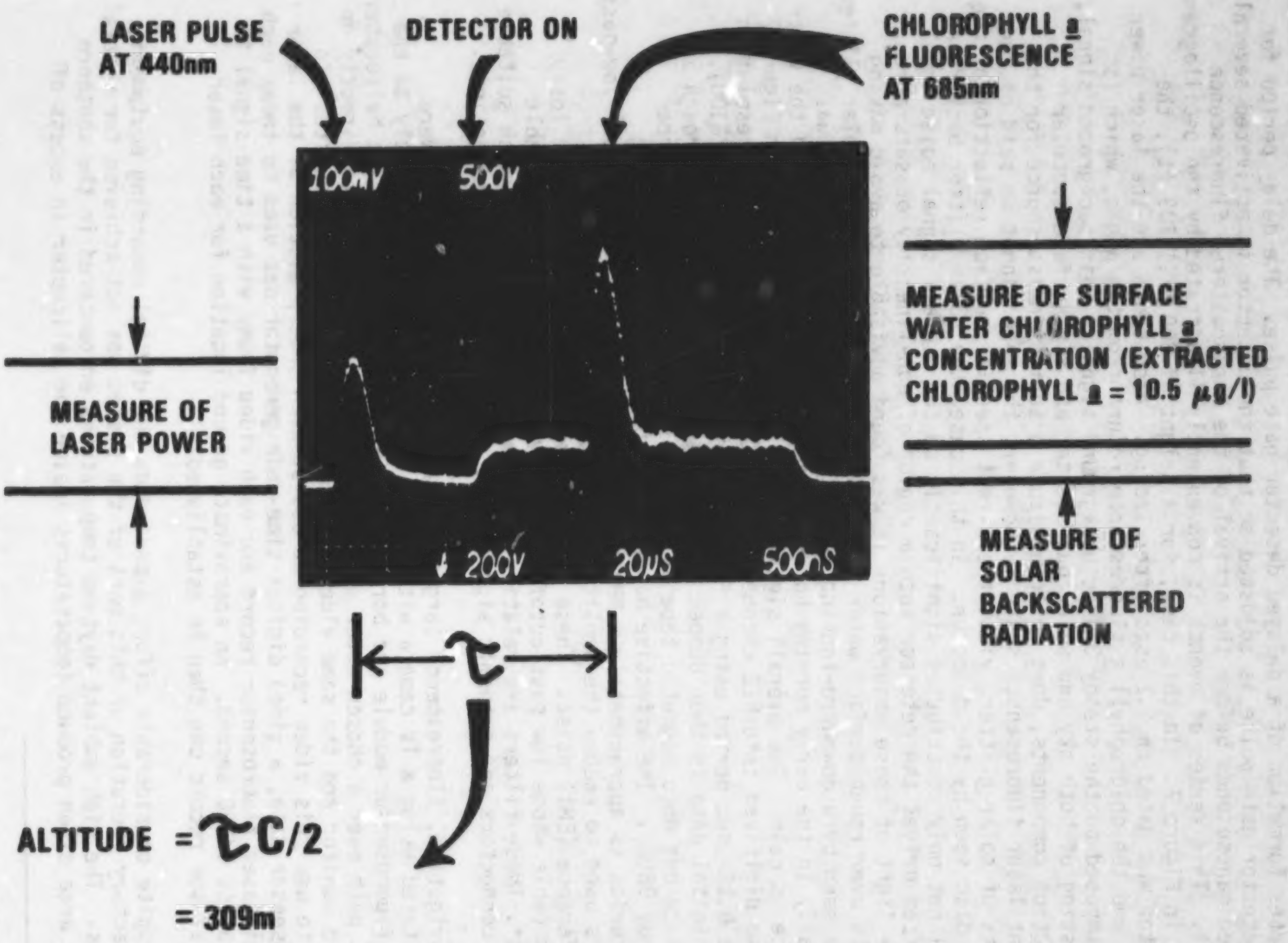

Figure 7. Oscillogram showing sequence of airborne laser-fluorosensor signals obtained over buoy #12 on October 4, 1976, for a measured chlorophyll a concentration of 10.5 μg/l.

120° F. This heat buildup invariably induces component failure, particularly in the digital electronic equipment. This problem has been temporarily solved by scheduling the test flights during the early hours of the morning when ambient temperatures are some 30° F to 40° F cooler than at midday. However, this expedient will not be acceptable when, of necessity, flights must be made at specific times of the day with a view to monitoring diurnal changes in the distribution of surface water algae. With these requirements in mind, it is planned to provide a dedicated cooling system for the digital rack-mounted instrumentation. Problems that were somewhat more difficult to eradicate concern the influence of vibrations and electronic noise on the performance of the digital recording system. Low-frequency vibrations in the region of 3 Hz to 7 Hz induced by the helicopter rotor blade were found to affect the precision alignment of the read and write heads of the digital tape recorder in relation to the tape transport assembly. This problem was eliminated by the addition of a stiffening member to the tape-drive support frame. Vibrations may also reduce the integrity of the many mechanical circuit connections in the digital equipment as exist with printed circuit board connector pins, wire-wrapped circuits and push-fitted integrated circuit chips. Although successful isolation from low-frequency vibrations in the region below 7 Hz is difficult to achieve using available aircraft-rated shock mount components, efforts are being made to improve the performance of the shock isolation rack mounting system used to hold the digital recording system. Finally, both the digital recording system and the monitoring oscilloscope have demonstrated a sensitivity to noise in the form of radio frequency interference (RFI) and EMI, particularly in the enclosed aircraft environment. Efforts are being made to improve the shielding of these components from external noise sources through the use of improved RFI screening, elimination of potential ground loops and additional filtering of the aircraft power supply. Due to the aforementioned problems with the digital equipment, it was not always possible to produce digital recordings of the airborne laser fluorosensor data. Consequently, oscillograms obtained at each sampling buoy location were used as the prime data source for comparison with the corresponding chlorophyll _a_ ground truth data.

# SECTION 7

# AIRBORNE MEASUREMENTS

## FIELD OPERATIONS

Airborne testing and evaluation of the laser fluorosensor were made over the Las Vegas Bay region of Lake Mead; a nautical chart of this area is shown in Figure 8. This bay is an ideal test site for a number of reasons. Firstly, it is located approximately 10 minutes flying time from McCarran (Las Vegas International) Airport. Secondly, this region has been extensively surveyed over a number of years by the Department of Biological Sciences, University of Nevada, Las Vegas (UNLV), because of the concern regarding the influence of nutrients on the population level of algae (34). High concentrations of nutrients and other pollutants enter the western end of Las Vegas Bay from Las Vegas Wash, which is essentially an open stream carrying both partially treated and untreated sewage water from the City of Las Vegas. At certain times of the year this pollution has been observed to support algal communities to the extent that chlorophyll a values will vary from 0.5 µg/l to 50 µg/l over a distance of 10 km. Finally, ground truth support for these airborne measurments was conveniently provided through the facilities and personnel of the Department of Biological Sciences at UNLV. A series of sampling station marker buoys positioned by the Department and the National Park Service are shown in Figure 8. This string of buoys, easily seen from a height of 200 m, was flown as three separate straight flight lines. The lengths of these lines from buoys 1 to 7, 7 to 11', and 11' to 12 are respectively 4452 m, 3544 m, and 1607 m. Not surprisingly, the highest chlorophyll a concentrations generally occurred in the region of buoy 12, which lies closest to the point where Las Vegas Wash enters Las Vegas Bay, whereas the lowest concentrations generally occurred at buoy 1. Lower chlorophyll a levels are encountered further out in the center of Lake Mead in the direction of Sentinel Island.

Airborne laser fluorosensor measurements were made over the three flight lines defined by buoys 1 to 7, 7 to 11' and 11' to 12 concurrent with collection of samples close to the buoys. Surface water grab samples were obtained at three different locations on the circumference of an approximately 25-m diameter circle around each sampling station in order to minimize the chances of collecting significant amounts of periphyton, which might break away from the sampling station buoy surface. These three samples were then mixed in order to minimize the effects of patchiness in the distribution of surface water algae.

When exciting chlorophyll a in the Soret absorption band region, the wavelength of the laser transmitter was tuned to lie close to 440 nm. Excitation at 440 nm avoids exciting phaeophytin a, a degradation product of

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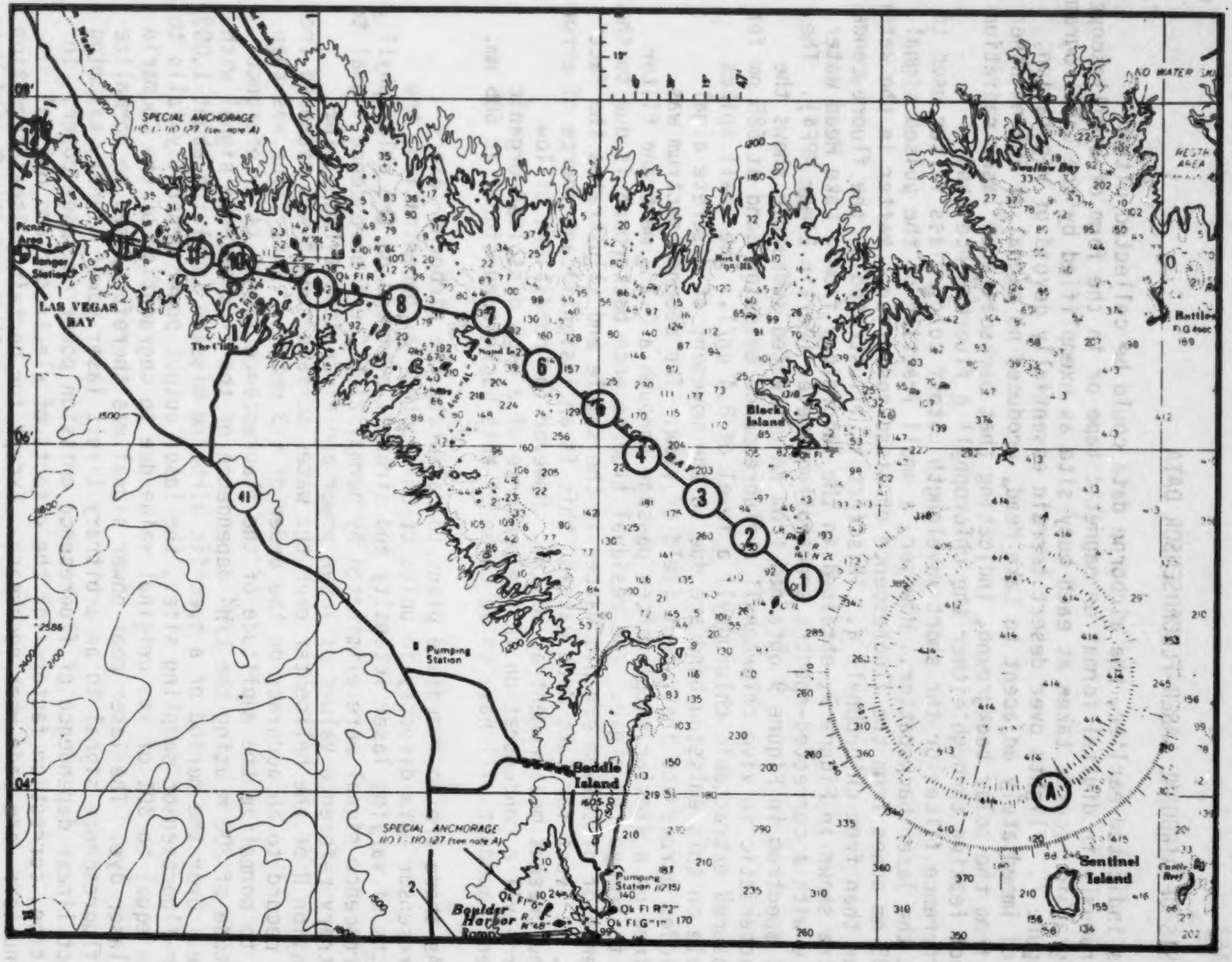
1
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A
LAS VEGAS BAY
SPECIAL ANCHORAGE
Black Island
Saddle Island
Sentinel Island
Boulder Harbor
Pumping Station
Swallow Bay
Battleship
NO WATER SKI
REST AREA
The Cliffs
Picnic Area
Ranger Station

Figure 8. Nautical chart of Las Vegas Bay region of Lake Mead, Nevada, showing location of marker buoy sampling stations.

chlorophyll a that has an excitation peak close to 420 nm but whose fluorescence cross section is approximately 10 times less than that of chlorophyll a at 440 nm when using excitation bandwidths smaller than 5 nm (35, 36).

## ANALYSIS OF AIRBORNE LASER-FLUOROSENSOR DATA

As indicated earlier, the airborne data could be collected either continuously in digital format on magnetic tape or in the form of oscilloscope photographic records taken at each buoy site as exemplified by the oscillogram in Figure 7. Flights over desert terrain essentially devoid of vegetation, located immediately adjacent to Lake Mead, produced no signal at 685 nm other than from the solar background, indicating that backscattered laser radiation was not leaking through either the chlorophyll a fluorescence band interference filter or the short wavelength cutoff color glass filter used to block the laser backscatter. However, a small fraction of the pulsed signal at 685 nm comes from the fluorescence of dissolved organic matter in the water rather than from chlorophyll a. This fact is illustrated by the fluorescence spectra shown in Figure 9, obtained in the laboratory from a Lake Mead water sample with a corrected-spectra spectrofluorometer (Perkin Elmer MPF4). The first spectrum in Figure 9 obtained from an untreated subsample shows the characteristic in vivo chlorophyll a fluorescence emission band at 685 nm for a measured extractable chlorophyll a level of 8.4 µg/l. The small spikes visible on this emission band are due to the movement of discrete algal particles across the instrument field of view. The second spectrum was obtained on a similar sample after passage through a 0.3 µ membrane filter used to remove the algae. The residual fluorescence background is due to the presence of dissolved organic matter in the sample and illustrates the fact that, at least for Lake Mead waters, this is an insignificant source of error to the present measurement at 685 nm. However for conditions of low chlorophyll a concentration in the presence of a high dissolved organic content, account will have to be taken of this background signal at 685 nm.

As there are no immediate plans to calibrate the airborne laser-fluorosensor data directly in units of chlorophyll a concentration, the effects of varying laser intensity and aircraft altitude on the chlorophyll a fluorescence signal were eliminated by normalizing the fluorescence signal to arbitrary reference values of laser power and aircraft altitude. The elevation H of the helicopter over the water surface target was measured from each record to an accuracy on the order of ± 3 meters. This value was then used to normalize the amplitude of the fluorescence signal to a reference altitude of 200 m using the $1/H^2$ dependence of the fluorescence signal with range. Over the period of a specific airborne mission involving about 1,000 laser-fluorosensor sampling sites, the laser output power generally falls to a value equal to 30% of its original value due to degradation of the Coumarin 120 laser dye. The laser peak power signal was therefore used to normalize the fluorescence signal to an arbitrary 1-volt laser power signal, assuming a direct linear dependence of fluoresence emission power on laser power. In addition, correction factors for the effects of electronic and optical attenuators were made when necessary. Except for certain pulse-integration and profile-smoothing procedures applied to the analysis of the digital data, interpretation of both analog (oscillographic) and digital data was

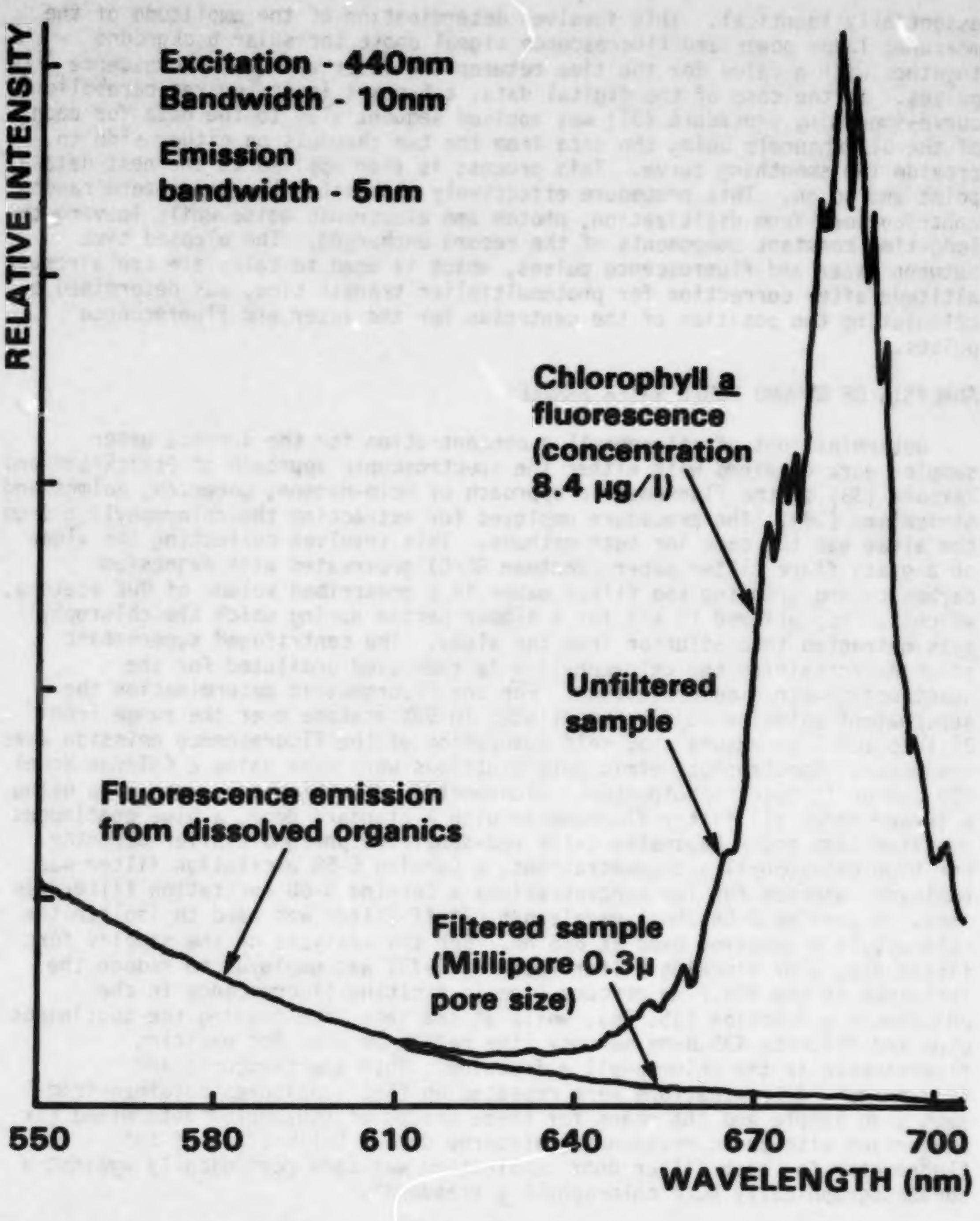

Figure 9. Corrected fluorescence emission spectra of filtered and unfiltered Lake Mead surface water sample, excited at 440 nm, August 16, 1977.

essentially identical. This involves determination of the amplitude of the measured laser power and fluorescence signal above the solar background together with a value for the time between the laser and the fluorescence pulses. In the case of the digital data, a 5-point least-squares parabolic curve-smoothing procedure (37) was applied sequentially to the data for each of the 512 channels using the data from the two channels on either side to provide the smoothing curve. This process is then applied to the next data point and so on. This procedure effectively eliminates the short-term random contributions from digitization, photon and electronic noise while leaving the long-time constant components of the record unchanged. The elapsed time between laser and fluorescence pulses, which is used to calculate the aircraft altitude after correction for photomultiplier transit time, was determined by calculating the position of the centroids for the laser and fluorescence pulses.

## ANALYSIS OF GROUND TRUTH WATER SAMPLES

Determinations of chlorophyll a concentration for the surface water samples were obtained with either the spectroscopic approach of Strickland and Parsons (38) or the fluorometric approach of Holm-Hansen, Lorenzen, Holmes and Strickland (39). The procedure employed for extracting the chlorophyll a from the algae was the same for both methods. This involves collecting the algae on a glass fibre filter paper (Whatman GF/C) pretreated with magnesium carbonate and grinding the filter paper in a prescribed volume of 90% acetone, which is then allowed to sit for a 3-hour period during which the chlorophyll a is extracted into solution from the algae. The centrifuged supernatant solution containing the chlorophyll a is then used undiluted for the spectrophotometric determination. For the fluorometric determination the supernatant solution is further diluted in 90% acetone over the range from 25:1 to 100:1 to ensure that self absorption of the fluorescence emission does not occur. Spectrophotometric determinations were made using a Coleman Model 620 Junior II Spectrophotometer. Fluorometric determinations were made using a Turner Model 111 filter fluorometer with a standard door, a blue continuous emission lamp and a Hamamatsu R-136 red-sensitive photomultiplier detector. For high chlorophyll a concentrations, a Corning 5-58 excitation filter was employed, whereas for low concentrations a Corning 5-60 excitation filter was used. A Corning 2-64 short-wavelength cutoff filter was used to isolate the chlorophyll a emission band at 685 nm. For the analysis of the samples for flight #12, a UV blocking filter (Corning 3-73) was employed to reduce the influence of the 404.7-nm mercury line in exciting fluorescence in the pheophytin a fraction (35, 36), while at the same time passing the continuous blue and discrete 435.8-nm mercury line radiation used for exciting fluorescence in the chlorophyll a fraction. Both spectroscopic and fluorometric determinations were repeated on five subsamples obtained from each grab sample and the means for these groups of subsamples determined for comparison with the corresponding airborne data. Calibration of the fluorometer for each filter door combination was made periodically against a chromatographically pure chlorophyll a standard*.

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*Pure chlorophyll a extract was obtained from Sigma Chemical Co., St. Louis, Mo.

An indication of the relative concentration of sample particulates (both algae and suspended sediment) was obtained using a 90° scattering nephelometer (Hach Model 2100A).

## COMPARISON BETWEEN AIRBORNE AND GROUND TRUTH DATA

Airborne laser fluorosensor data for flights #4 and #8, obtained using a laser excitation wavelength of 440 nm and taken about 1 month apart, are shown in Figures 10 and 11 respectively, together with the corresponding chlorophyll a ground truth data obtained by the spectroscopic method of Strickland & Parsons (38).

Elevation above the water surface was about 200 m for both flights. Since absolute calibration of the airborne fluorescence data in terms of chlorophyll a concentration was not provided, it was found convenient to normalize each airborne data set to the corresponding chlorophyll a ground truth data set by minimizing the sum of the squares of the differences between the corresponding airborne and ground truth values (least squares procedure).

Changes in the 10-kilometer (km) long surface water profiles, for both the airborne and ground truth measurements for flight #4, shown in Figure 10, appear to be in general agreement. Comparison between the airborne and ground truth data for this flight gave a linear correlation coefficient of 0.95. The data for flight #8 made over the same path on a later date exhibit a somewhat lower correlation coefficient of 0.77. It is apparent that some of the large point-to-point fluctuations present in the ground truth profile for this flight are also present in the airborne fluorescence profile, though in a somewhat attenuated form. Similar but smaller fluctuations were also present in the fluorometrically determined chlorophyll a data for flight #8 which, when compared to the laser fluorosensor data, gave a correlation coefficient of 0.85. Linear correlations between airborne and ground truth data for these and other flights are presented in Table 3 with the values lying in the range from 0.41 to 0.95. For a sample size of 12 or larger, with $r \geq 0.57$, correlations are significant at the 5% level whereas with $r \geq 0.70$, they are significant at the 1% level. It is interesting to note that the correlation ($r=0.41$) for the data of flight #12 obtained using a laser fluorosensor excitation wavelength of 622 nm is not significant at the 10% level. One of the reasons for using this excitation wavelength was to investigate the correlation between the airborne data and the biomass for specific algal color groups, and in particular for blue-green algae. Further discussion on the data for this flight is deferred to Section 7f. Also shown in Table 3 are the correlation coefficients relating the laser fluorosensor data to that for water turbidity obtained using a 90° scattering nephelometer. It is significant that, in all cases, these coefficients are only slightly less than those for the correlation between the laser fluorosensor data and that for the spectroscopically determined chlorophyll a. This suggests, at least from an optical viewpoint, that the particulate matter in surface waters of Las Vegas Bay consists principally of algae rather than of suspended sediment.

An estimate of the sensitivity limit for the laser fluorosensor in its present form can be gauged from fluorescence pulse data provided by the oscillogram in Figure 7. Assuming that for daytime operation, system

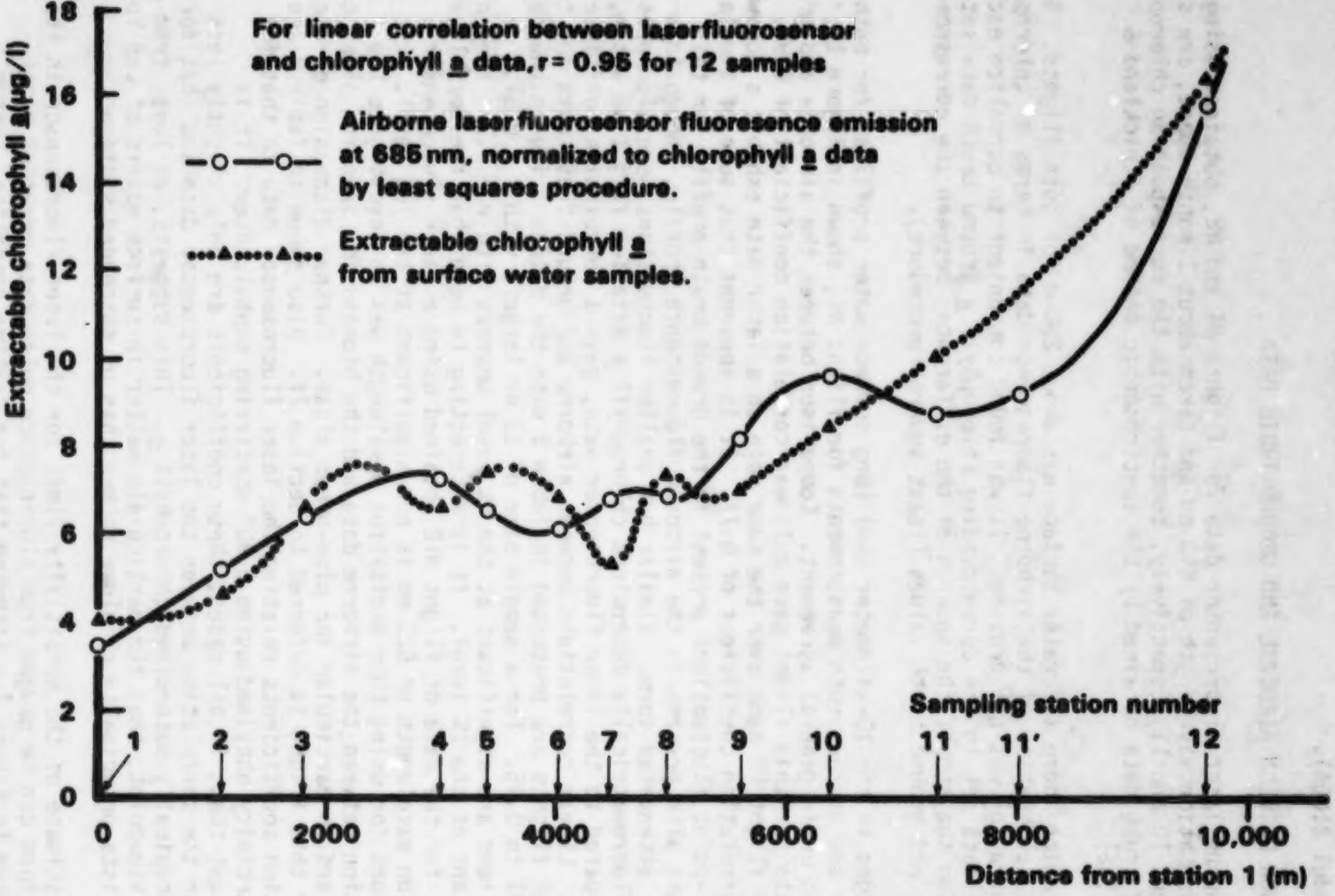

Figure 10. Variation of surface water chlorophyll a and laser-fluorosensor signal with distance for surface water transect of Las Vegas Bay in Lake Mead, Nevada, Flight #4, October 15, 1976.

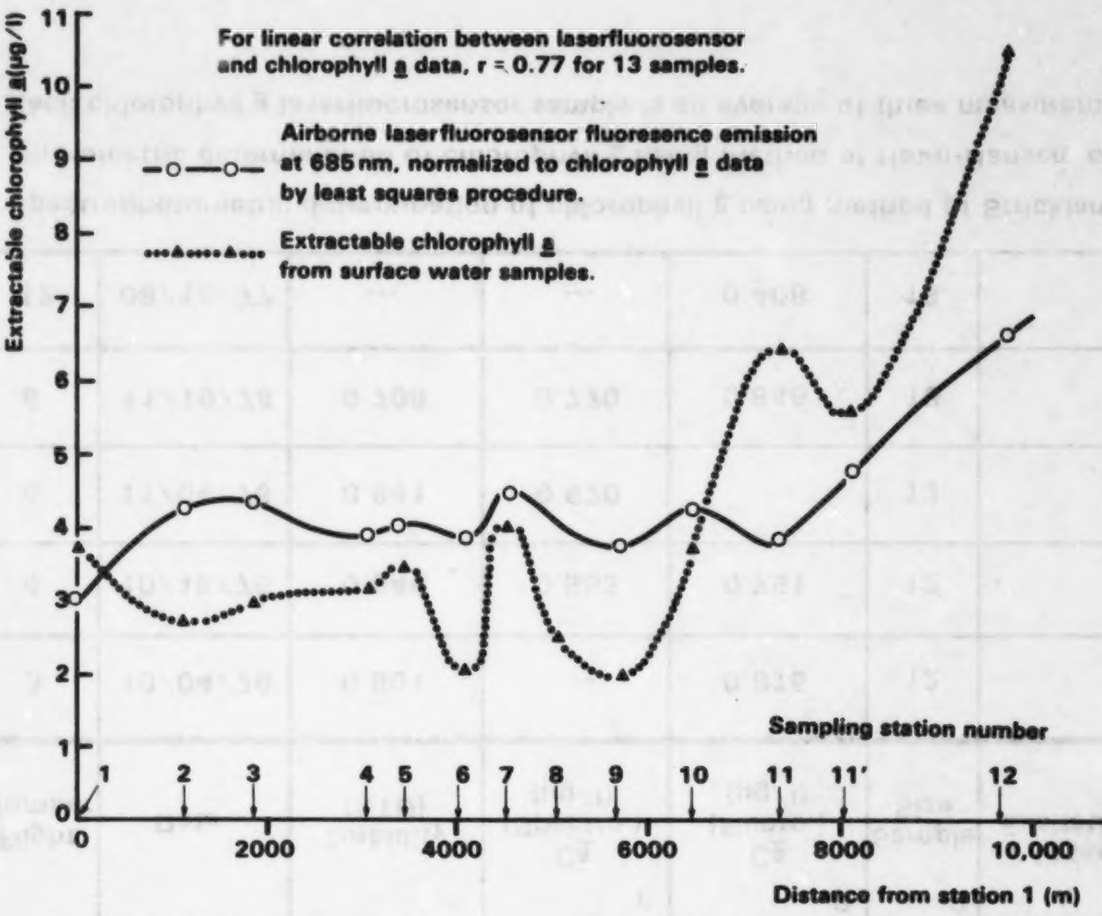

Figure 11. Variation of surface water chlorophyll a and laser-fluorosensor signal with distance for surface water transect of Las Vegas Bay in Lake Mead, Nevada. Flight #8, November 19, 1976.

TABLE 3. CORRELATION COEFFICIENTS FOR CORRECTED LASER-FLUOROSENSOR SIGNAL VERSUS TURBIDITY AND CHLOROPHYLL a DATA

| Flight Number | Date | Turbidity (NTU) | A<br>Ca (Spectro.) (μg/l) | B<br>Ca (Fluoro.) (μg/l) | C<br>Sample Size | Laserfluorosensor Excitation Wavelength (nm) |
|---|---|---|---|---|---|---|
| 3 | 10/04/76 | 0.801 | --- | 0.815 | 12 | 440 |
| 4 | 10/15/76 | 0.846 | 0.953 | 0.751 | 12 | 440 |
| 5 | 11/04/76 | 0.841 | 0.870 | --- | 13 | 440 |
| 8 | 11/19/76 | 0.708 | 0.770 | 0.846 | 13 | 440 |
| 12 | 08/16/77 | --- | --- | 0.408 | 13 | 622 |

A) Spectrophotometric determination of chlorophyll a using method of Strickland and Parsons (38).

B) Fluorometric determination of chlorophyll a using method of Holm-Hansen et al. (39).

C) Each chlorophyll a laserfluorosensor sample is an average of three measurements.

sensitivity is limited by the backscattered sky solar background radiation existing within the chlorophyll a fluorescence band, a peak Signal to RMS (Root Mean Square) Background Noise Ratio (SBNR) can be calculated using

$$\mathrm{SBNR} = V_p / v_{RMS}$$

where $V_p$ is the steady peak detector voltage of the fluorescence pulse above the steady background level and $v_{RMS}$ is the RMS noise voltage of the background signal. Based on the fact that shot or photoelectron noise is white noise with an instantaneous Gaussian probability distribution, a statistical criterion can be adopted whereby the peak to peak fluctuations of the background lie within a total spread of 5 standard deviations for 99% of the time. Because one standard deviation is defined as the RMS noise voltage $v_{RMS}$, we can write the equality

$$v_{PP} = 5\ v_{RMS}$$

where $v_{PP}$ is the peak-to-peak spread in the noise envelope for 99% of the time. The expression for the peak signal to RMS background noise ratio then becomes

$$\mathrm{SBNR} = 5\ V_p / v_{PP}$$

As depicted in Figure 7, $V_p = 0.64$ volts for a chlorophyll a concentration of 10.5 µg/l and $v_{PP} = 0.04$ volts; it follows that for a minimum acceptable SBNR of 3, the system can monitor chlorophyll a levels down to 0.4 µg/l.

This sensitivity limit is subject to a number of qualifiers. First, these measurements were made under clear skies with the sun near the zenith. With a water albedo on the order of 0.05, the noise due to the solar backscatter (but not surface glitter), was at the high end of the anticipated range. However, albedo values in the range from 0.2 to 0.3, due to high concentrations of suspended sediment, would substantially increase this background noise. Second, it is assumed that the contribution to the laser induced fluorescence signal at 685 nm due to dissolved organics is negligible. For the surface waters of Lake Mead, the amplitude of the fluorescence signal at 685 nm due to dissolved organics has an equivalent chlorophyll a value of about 0.1 µg/l. For water conditions where this is not the case, an estimate of this background signal must be obtained by making an additional laser-fluorosensor spectral measurement, say in the region of 650 nm. Third, the present sensitivity limit corresponds to water conditions for which the optical transmission is dominated by algal particles. For conditions where suspended sediment is the principal factor limiting transmission, system sensitivity will be reduced in relation to the values indicated above. This situation can be expected to exist for suspended sediment concentrations greater than 50 mg/l.

A number of expedients can be employed to improve system sensitivity. Increasing the laser energy or peak power will directly increase $V_p$, whereas reducing the laser beam divergence, so that the receiver collects a smaller solar background signal, will reduce $v_{PP}$. The background noise signal $v_{PP}$ can also be reduced to near zero by flying at dawn or dusk or during nighttime, in contrast to the present experiments, which were flown close to midday under clear skies but in the absence of significant solar glitter. In this situation, system sensitivity is limited by "in-signal" photoelectron noise. In any case, to achieve a system sensitivity limit down to a chlorophyll a concentration of 0.1 μg/l, with a signal-to-noise ratio of 10 should represent no major technical problems. This level of sensitivity, in effect, requires a 13-1/3-fold increase in the overall SBNR over the performance of the present system. With a larger laser flashlamp and increased laser input energy, a doubling in the laser output power and energy can be achieved with a corresponding 2-fold increase in the SBNR (31). Similarly a 1.77-fold-enhancement in the SBNR can be achieved by increasing the FWHM-bandwidth of the optical filter at 685 nm from its present value of 13 nm to 23 nm, so as to be in close correspondence with the width of the chlorophyll a band. Further, by utilizing a telescope with a reflecting element rather than a refracting element, the circle of least confusion (the blur circle) caused by spherical aberration can be reduced from the present 6-mm-diameter to a size much smaller than the 2-mm-diameter image of the surface water laser spot. A 2-mm-diameter focal plane stop could then be used rather than the present 4-mm-diameter stop, thereby reducing the magnitude of the background signal and noise without affecting the magnitude of the chlorophyll a fluorescence signal. This measure produces an additional 2-fold enhancement in the SBNR (31). Finally, by halving the elevation of the airborne platform over the water surface target, the chlorophyll a fluorescence signal would be enhanced by a factor of 4 based on the $1/H^2$ dependence of the fluorescence signal on altitude; this expedient does not affect the magnitude of the background signal for an extended homogeneous target. This procedure in turn produces a 4-fold increase in the SBNR (31). With implementation of all of the aforementioned system modifications, the SBNR would be increased overall by a factor 27 times the present value, thereby realizing a SBNR of 20 at a chlorophyll a level of 0.1 μg/l under the environmental conditions of the present measurements.

It should be noted that reducing the aircraft height also reduces the laser spot size, thereby increasing the sensitivity of the laser-fluorosensor signal to small-scale variations in the concentration of surface water algae. As indicated in Section 4, this can be accommodated for by increasing the laser repetition rate.

## FACTORS INFLUENCING THE RELATIONSHIP BETWEEN AIRBORNE AND GROUND TRUTH DATA

Several explanations exist for the less than perfect covariation between the laser fluorosensor signal and the corresponding surface water chlorophyll a determinations. Factors influencing these correlations are either random or systematic in origin and are discussed separately.

Random errors compounded by a small sample size of 12 or 13 will clearly reduce the degree of correlation. Differences are known to exist between the locations at which the grab samples were obtained and those at which the airborne measurements were made for any one sampling station. Uncertainties on the order of 50 m combined with significant patchiness in the surface water algae distribution can be expected to degrade any such correlation. With a view to minimizing this source of error, samples were collected at three different locations on the circumference of an approximately 25-m diameter circle around each sampling station buoy and then mixed prior to analysis. At the present time, it is not clear what residual error is present due to differences between the airborne and ground truth sampling locations. Direct grab sampling from the helicopter would eliminate this problem although the helicopter rotor down-wash might induce considerable horizontal mixing and water movement at the sampling site. Random errors are also incurred in the measurement of the extractable chlorophyll a. The established method for extracting chlorophyll a from algae in water samples (40) by filter grinding and extraction in 90% acetone is known to be both inefficient and subject to considerable variability due to the production of chlorophyll a degradation products (41, 42). For a total of five determinations made on each grab sample, coefficients of variation ($s/\bar{x}$) of up to ±50% have been observed for chlorophyll a levels down to 1 μg/l. More efficient and reproducible methods for extracting chlorophyll a from algae are presently under investigation and will be reported elsewhere.

Systematic errors are best discussed in terms of the laser fluorosensor equation (Equation 1) for remote monitoring of in vivo chlorophyll a. The environmental factors within the fourth set of square brackets in Equation 1 are considered to be the primary source of systematic error encountered with the laser-fluorosensor technique. Specifically, the discussion centers on the validity of the assumptions that the fluorescence cross section $\sigma_C$ and the water diffuse attenuation coefficients $k_L$ and $k_F$ remain constant for all surface water locations.

As discussed in the Review, $\sigma_C$, is dependent on a number of factors including temperature, nutrient and pollutant-induced stress, and solar radiation intensity levels, as well as on the intensity, wavelength and duration of the excitation source. More significant is the dependence of $\sigma_C$ on the type and relative concentration of the various algal color groups present in the water surface at any one time and place. In reality, all of the photopigments will contribute to the fluorescence emission at 685 nm. Their individual contributions will depend on the nature of the excitation spectrum for the different algal color groups present, their relative concentration, and the excitation wavelength employed. Under these circumstances, it is clear that the concept of a single unique overall value for $\sigma_C$ is invalid, a fact that possibly provides, at least in part, an explanation for the variation in the correlation coefficients obtained between the airborne and ground truth data on different occaqions, as shown in Table 3. Ideally, the actual value of $\sigma_C$ for every airborne sample site should be determined by making laboratory fluorometric measurements on grab samples with the appropriate excitation wavelength. By measuring $n_C$, $k_L$, $k_F$, $P_L$ and $P_F$, and also knowing all the other system and environmental factors, a representative value of $\sigma_C$ can then be established for a given water sample through use of Equation 1, regardless of the photosynthetic pigments contributing to the fluorescence emission at 685 nm. The impracticality of such an approach for a remote sensing application is obvious. An alternative and more attractive approach entails finding an excitation wavelength for which the individual fluorescence cross sections for the different algal color groups are all equal or at least of a similar order of magnitude. As mentioned in Section 4, published data (15, 16, 25) suggest the use of wavelengths in the 600-nm to 620-nm region. However more recent measurements made on algal monocultures representative of the different color groups (21) indicate that the fluorescence excitation cross section at 618 nm for a blue-green algal monoculture (anacystis marinus) was, on the average, about 5 times larger than the values for the other color groups. Unfortunately, as mentioned in Section 7 (d), the present airborne measurements, made by using an excitation wavelength of 622 nm, were not successful (the laser fluorosensor and chlorophyll a ground truth data were poorly correlated, with $r = 0.41$). However, as will be discussed in more detail in Section 7 (f), the reason for this poor correlation is related primarily to faulty ground truth chlorophyll a determinations rather than to an inherent weakness in the laser fluorosensor method. A final decision on the choice of an optimum excitation wavelength must therefore await further tests of the airborne laser fluorosensor. At that time it will then be possible to pass judgement on the validity of the concept of using a single unique overall value of $\sigma_c$ for characterizing the fluorescence of in vivo chlorophyll a at a given excitation wavelength.

Inspection of Equation 1 shows that changes in the surface water values for $k_L$ and $k_F$, the diffuse attenuation coefficient at the laser and fluorescence wavelengths respectively, directly influence the laser-fluorosensor determination of $n_c$, regardless of whether a relative or absolute value is required. The assumption was made that changes in concentration of particulate and dissolved matter were sufficiently small over the extent of a given water surface that $k_L$ and $k_F$ could be considered be constant. This premise in turn requires the assumption that optica effects caused by algal scattering and absorption contribute a negligible percentage to the total values for $k_F$ and $k_L$. However, situations exist where the related beam attenuation coefficient $\alpha_\lambda$ is highly correlated with either extracted chlorophyll a or in vivo chlorophyll a fluorescence.

Kiefer and Austin (28) obtained linear correlation coefficients of 0.95 for variation of the beam attenuation coefficient $\alpha_\lambda$ versus chlorophyll a fluorescence and of 0.95 for $\alpha_\lambda$ versus $n_c$, the concentration of extracted chlorophyll a. These measurements were made in a marine environment for chlorophyll a values ranging from 1 µg/l to 6 µg/l. The least-squares best-fit line for the latter correlation is given by

$$\alpha_\lambda = 0.99 + 0.2\, n_c \tag{2}$$

where the $\alpha_\lambda$ values have been corrected for the component of attenuation due to optically pure seawater at wavelength $\lambda$. Similar measurements by Baker and Baker (29), made in fresh water environment, realized a correlation coefficient of 0.83 for uncorrected $\alpha_\lambda$ values versus extracted chlorophyll a levels up to 100 µg/l. The least-squares best-fit line for this data set is

$$\alpha_\lambda = 3.57 + 0.11\, n_c \tag{3}$$

From these measurements, it would appear that, for extracted chlorophyll a levels at least greater than 1 µg/l, a linear relationship exists between extracted chlorophyll a with a slope factor lying in the range from 0.1 $m^{-1}$/µg/l to 0.2 $m^{-1}$/µg/l. For surface water samples containing high and variable concentrations of non-algal matter in addition to that for the chlorophyll-related pigments, this relationship can be expected to break down due to the unpredictable contribution to the overall attenuation coefficient from this non-algal matter. This effect is further exacerbated by the fact that algal population growth can become arrested or even reversed by the restrictions put on photosynthetic activity by the reduction in available solar radiation caused by high concentrations of suspended sediment.

Confirmation of a relationship between $n_C$ and $\alpha_\lambda$ for the surface waters of Las Vegas Bay was obtained by making a concurrent measurement of in vivo chlorophyll a fluorescence and the beam attenuation coefficient at 610 nm. An in situ measurement of the beam attenuation coefficient was obtained using a 1-meter path length transmissometer (Martek Model XMS). This device, which measures beam transmittance T with a collimated and filtered light source, provides values of $\alpha_\lambda$ from the expression

$$T = \exp(-\alpha_\lambda x)$$

where x (= 1 m) is the optical path length of the beam. The transmissometer was rigidly mounted to the bow of the survey launch at a depth of about 1 meter and, as such, provided a continuous chart-recorded profile of the surface water optical transmission. The present measurements were made at a wavelength of 610 nm with a filter with a half-height bandwidth of 32 nm, so that this transmission measurement is a close approximation for $k_F$, the diffuse attenuation coefficient for the chlorophyll a fluorescence emission wavelength at 685 nm. The concurrent in vivo chlorophyll a fluorescence profile was made using a filter fluorometer (Turner Model 111) as described in Section 7 (c), except that a high volume flow-through sample cell was used in place of the standard cuvette. The entry port for the flow-through sample tube was located directly adjacent to the optical path of the transmissometer with a sample pump located downstream of the fluorometer in order to avoid damage or contamination of the sample by either cavitation bubbles or pump lubricant. The continuous in vivo fluorescence and optical transmission profiles obtained for the region in Las Vegas Bay between stations 9 and 12 are shown in Figure 12. The in vivo fluorescence data were calibrated in terms of equivalent extracted chlorophyll a through use of the single chlorophyll a determination performed on a sample obtained at station 11, as indicated by the single reference point in the chlorophyll a profile.

The discrete $\alpha$ values, plotted for equal intervals of time, were calculated directly from the corresponding 1-meter path length transmission profile data using Equation 4. The variations in the attenuation coefficient data are in almost complete correspondence with those for the chlorophyll a fluorescence data, after corrections are made for the approximately 6-second time delay in the fluorometric data. This delay represents the finite time needed to pump a sample from the location of the submerged transmissometer to the deck location of the fluorometer. The same discrete attenuation coefficient values are also shown in Figure 13 plotted against the corresponding in vivo chlorophyll a values obtained from the calibrated profile in Figure 12; a linear correlation coefficient of 0.96 was obtained from this group of 25 data points. Extrapolating the curve to a zero fluorescence value suggests a background attenuation coefficient value of about 1.7 $m^{-1}$, due principally to dissolved and non-algal particulate

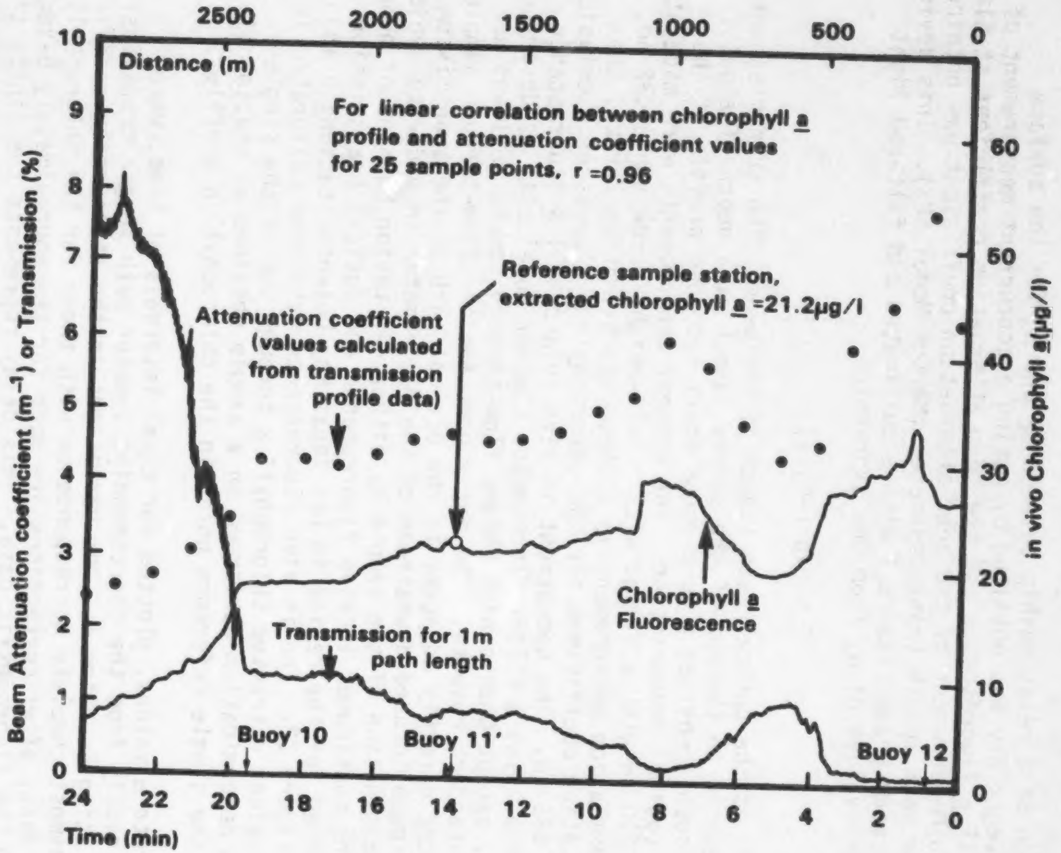

Figure 12. In vivo chlorophyll a fluorescence and water transmission profiles obtained from surface water transect of Las Vegas Bay in Lake Mead, Nevada, June 8, 1977. Also shown are beam attenuation coefficient values calculated for 25 points in the transmission profile. Transmission data measured at 610 nm.

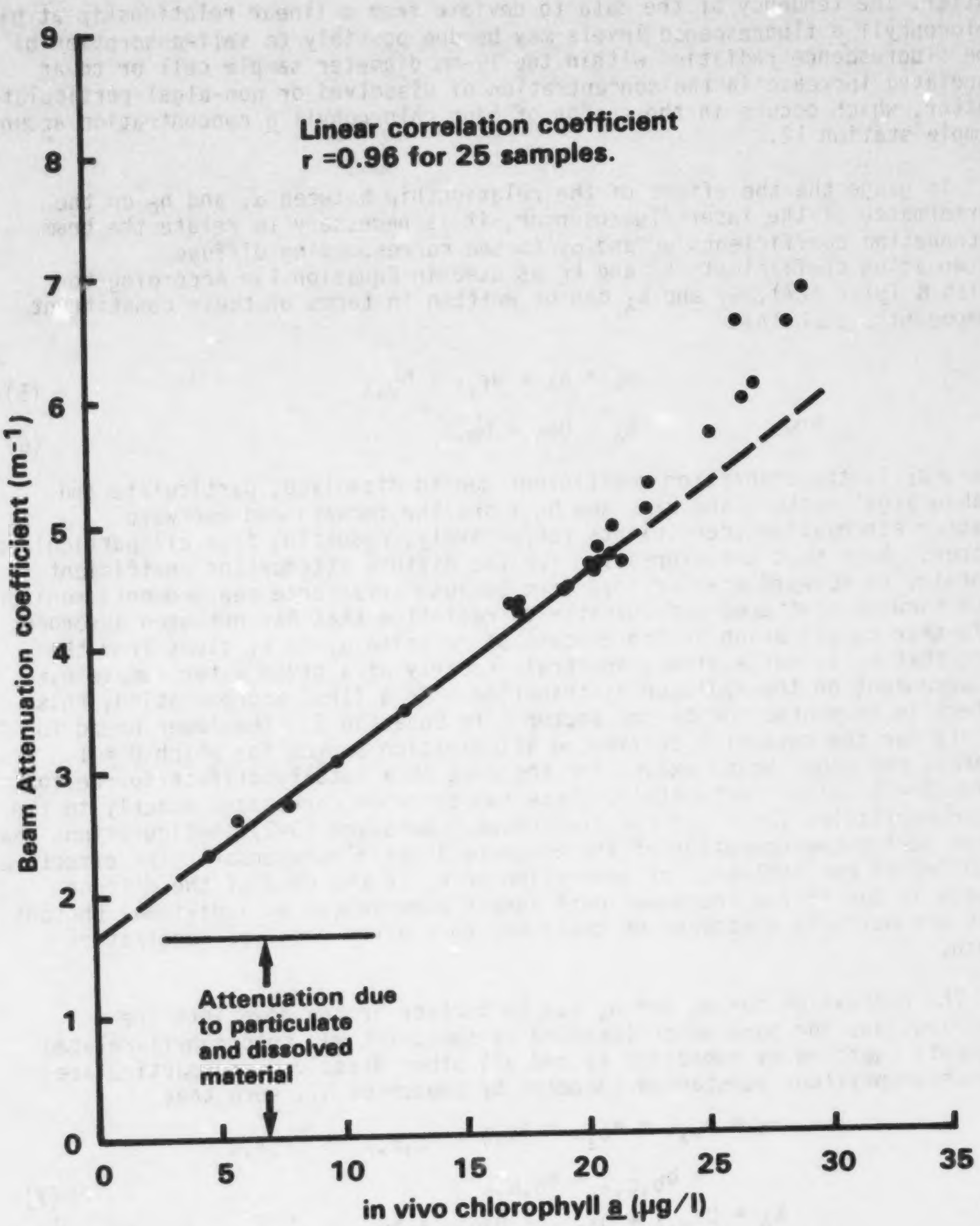

Figure 13. Variation of beam attenuation coefficient with in vivo chlorophyll a fluorescence for surface water transect of Las Vegas Bay in Lake Mead, Nevada, June 8, 1977. Attenuation data measured at 610 nm.

matter. The tendency of the data to deviate from a linear relationship at high chlorophyll a fluorescence levels may be due possibly to self-absorption of the fluorescence radiation within the 19-mm diameter sample cell or to an unrelated increase in the concentration of dissolved or non-algal particulate matter, which occurs in the region of high chlorophyll a concentration around sample station 12.

To gauge the the effect of the relationship between $\alpha_\lambda$ and $n_c$ on the performance of the laser fluorosensor, it is necessary to relate the beam attenuation coefficients $\alpha_L$ and $\alpha_F$ to the corresponding diffuse attenuation coefficients $k_F$ and $k_L$ as used in Equation 1. According to Smith & Tyler (27), $\alpha_\lambda$ and $k_\lambda$ can be written in terms of their constituent components, such that

$$\alpha_\lambda = a_\lambda + b_{f,\lambda} + b_{b,\lambda} \tag{5}$$

and

$$k_\lambda = Da_\lambda + b_{b,\lambda} \tag{6}$$

where $a_\lambda$ is the absorption coefficient due to dissolved, particulate and viable algal matter, and $b_{f,\lambda}$ and $b_{b,\lambda}$ are the forward and backward scatter attenuation coefficients respectively, resulting from all particulate matter. Note that the expression for the diffuse attenuation coefficient contains no forward scatter loss term because irradiance measurements monitor both forward scattered and unscattered radiation that has not been absorbed. A further complication in the process of relating $\alpha_\lambda$ to $k_\lambda$ stems from the fact that $k_\lambda$ is not a simple spectral property of a given water sample but is dependent on the radiance distribution. As a first approximation, this effect is accounted for by the factor D in Equation 6. The lower bound for D exists for the case of a collimated illumination source for which D = 1 whereas the upper bound exists for the case of a totally diffuse source for which D = 2 (27). Fortunately, these two extremes correspond exactly to the laser excitation (D=1) and the fluorescence emission (D=2) configurations that occur during the operation of the airborne laser fluorosensor. The effective doubling of the influence of absorption on $k_\lambda$ in the case of the diffuse source is due to the increased path length experienced by individual photons that are multiply scattered on their way to a given vertical penetration depth.

The expression for $k_\lambda$ and $\alpha_\lambda$ can be further broken down into the contributions for pure water (denoted by subscript w), chlorophyll-related pigments (denoted by subscript c) and all other dissolved and particulate non-chlorophyllous substances (denoted by subscript N), such that

$$\alpha_\lambda = a_{w,\lambda} + a_{c,\lambda} + a_{N,\lambda} + b_{f,c,\lambda} + b_{f,N,\lambda} + b_{b,c,\lambda} + b_{b,N,\lambda} \tag{7}$$

and

$$k_\lambda = Da_{w,\lambda} + Da_{c,\lambda} + Da_{N,\lambda} + b_{b,c,\lambda} + b_{b,N,,\lambda} \tag{8}$$

where the molecular (Rayleigh) scattering contributions from pure water are considered to be negligible and have been omitted.

Finally a linear expression can be given for $\alpha_\lambda$ in terms of $n_C$ as typified by the data given in Figure 13, such that

$$\alpha_\lambda = A_\lambda + B_\lambda n_C \tag{9}$$

where $A_\lambda$ and $B_\lambda$ are presumed to be constant for a given locality and wavelength λ. For the laser excitation case where D=1, Equations 7, 8 and 9 can be solved to give $k_L$ in terms of measurable parameters $A_L$ and $B_L$ and the scattering coefficients $b_{f,c,L}$ and $b_{f,N,L}$, where

$$k_L = B_L n_C + A_L - b_{f,c,L} - b_{f,N,L} \tag{10}$$

Similarly for the fluorescence emission case with D = 2, Equations 7, 8 and 9 provide a similar expression for $k_F$ such that

$$k_F = B_F n_C + A_F - b_{f,c,F} - b_{f,N,F} + a_{w,F} + a_{c,F} + a_{N,F} \tag{11}$$

Substituting the expressions for $k_L$ and $k_F$, given by equations 10 and 11 respectively, into Equation 1, we have

$$n_C = \left[\frac{P_F}{P_L} H^2\right]\left[\frac{4\pi\Delta_F}{T\eta_{Tr}\eta_{Rec}}\right]\left[\frac{\mu_W^2 \exp\{H(\beta_L + \beta_F)\}}{(1-R_W)^2}\right]\left[\frac{1}{\sigma_C}\right]\left[X_C + X_N\right] \tag{12}$$

$X_C$, which represents the optical loss terms due to the chlorophyll pigments, is given by

$$X_C = \left[n_C (B_L + B_F) + a_{c,F} - (b_{f,c,L} + b_{f,c,F})\right] \tag{13}$$

Although the terms $b_{f,c,L}$ and $b_{f,c,F}$ within the expression for $X_C$ are not given explicitly in terms of $n_C$, it is reasonable to assume a linear dependence such that $X_C$ will also vary as $n_C$. $X_N$, which represents all other optical loss terms, is given by

$$X_N = \left[A_L + A_F + a_{N,F} + a_{w,F} - (b_{b,N,L} + b_{f,N,F})\right] \tag{14}$$

From this expression, it is clear that we no longer have a simple relationship in which $P_F$ varies linearly as $n_C$, but rather one in which the additional factor $X_C$ is also dependent on $n_C$. To monitor relative changes in $n_C$, it now becomes necessary to obtain exact values for all of the other terms in addition to $(P_F/P_L)H^2$, in particular for the chlorophyll a fluorescence cross section $\sigma_C$, and the various chlorophyll and non-chlorophyll optical loss terms represented by $X_C$ and $X_N$ respectively. The determination of values for all of these unknown parameters at each surface water sampling point is clearly not feasible, particularly from a remote sensing viewpoint. Two limiting cases of Equation 12 are of interest. First, when absorption and scattering losses from non-algal sources are dominant, such that $X_N/X_C >> 1$, the term $X_C$ can be omitted so that the attenuation coefficients are no longer dependent on $n_C$. A situation then exists in which $n_C$ varies as $(P_F/P_L)H^2$; this is the situation which was assumed to exist in the derivation of Equation 1 by Browell (20). A different situation exists however when scattering losses arise principally from the presence of algae such that $X_C/X_N << 1$. In this case, $n_C$ can be eliminated from both sides of Equation 12 and the laser fluorosensor measurement represented by the term $(P_F/P_L)H^2$ remains constant for all values of $n_C$. Put in physical terms, the lower the concentration of algae or equivalent extractable chlorophyll a, the larger the penetration depth of the laser beam. In turn this situation means that the laser beam can ultimately intercept the same total algae count it that would have encountered in high algae count situations. As a consequence, the received chlorophyll a fluorescence power will remain constant, independent of changes in $n_C$. In reality, the truth will lie somewhere in between these two extremes depending upon whether optical losses are dominated by algal or non-algal matter.

Because a direct airborne measurement of the absolute values for $k_L$ and $k_F$ is not possible within the framework of the present program, an alternative approach must be considered. A possible means for monitoring changes in $k_\lambda$ and, in particular, its dependence on $n_C$ can be provided by obtaining a relative indication of laser beam penetration through the surface water layer by concurrently monitoring the water Raman emission signal. As the Raman emission is a property of the water alone, the intensity of the observed signal will depend only on $k_\lambda$ and hence only indirectly on $n_C$. Changes in the water Raman emission signal can therefore be used to provide information on changes in $k_\lambda$. A more extensive discussion of this method will be presented in Section 8.

On the basis of the foregoing discussion, the relative contributions of variations in $\sigma_C$, $k_L$ and $k_F$ to the degradation of the correlation between the airborne and ground truth data are not clear. With implementation of the Raman technique for correcting for changes in $k_L$ and $k_F$, it should be possible to isolate the residual uncertainty due to $\sigma_C$. The effects of variability in $\sigma_C$ on the correlation between the airborne and ground truth data can then be minimized by finding an excitation wavelength for which the fluorescence cross sections for the different algal color groups are more nearly equal.

## REMOTE MONITORING OF BLUE-GREEN ALGAE

In fresh waters, blue-green algae (cyanophyta) are the only algal division that contains the photopigment c-phycocyanin. Radiation absorbed by this pigment, which has an excitation peak close to 622 nm, is transferred internally into the chlorophyll a photosynthetic system. The small fraction of this radiation that leaks out as chlorophyll a fluorescence at 685 nm is employed as an indicator of the relative concentration of blue-green algae present in a given surface water sample. This approach assumes that the fluorescence cross section for all possible mixtures of all species of blue-green algae present at a specific location at a given time remains constant with a value at least 10-fold larger than that for the combined effect of all other color groups.

An airborne experiment was conducted employing laser excitation at 622 nm rather than at 440 nm, with the purpose of selectively exciting fluorescence in the blue-green algae. Variations in the biomass for each algal division, extractable chlorophyll a, and the laser fluorescence signal as measured over the established Las Vegas Bay flight line, are shown in Figure 14. A further comparison of these data is presented in Table 4 in the form of a matrix of linear correlation coefficients relating biomass from each algal division to either extractable chlorophyll a or the laser fluorsensor signal. Biomass determinations were made by collecting the algae from a given sample volume onto a Millipore HA 0.45μ membrane filter. Enumeration for all species present in significant numbers was made with a microscope at either 400X power or 1000X power. At the latter magnification, the filters were soaked in a refractive index matching fluid to aid in the identification process. Algal counts were made for 10 Whipple fields for each filter and a volume established for each algal species. From these data, biomass in mg/l was calculated for each algal division or color group assuming a constant algal density of 1. Finally, for each sample, an estimate of total algal biomass was obtained by summing the contributions from each algal division.

Figure 14 shows that the laser fluorosensor signal progressively increases toward station 12 in contrast to the blue-green algal biomass determination, which shows a slight downward trend. The correlation coefficient for covariation between these parameters was -0.48, which is significant at the 10% level. Although no straightforward explanation exists for an apparent inverse dependence of this kind, a number of interfering effects might be responsible. The laser fluorosensor signal appears to follow more closely the trends in the total biomass determination for which the correlation coefficient has a value of +0.82, significant at the 1% level. This observation suggests that excitation radiation at 622 nm is being absorbed directly by the chlorophyll a present in all algae as well as by c-phycocyanin common only to blue-green algae. This conclusion is supported by data presented by Mumola, Jarrett and Brown (16). They show that, although the excitation spectra for blue-green algae peaks close to 618 nm, its absolute value given in terms of its fluorescence cross section differs only slightly from the values for the other algal color groups at 618 nm. In this respect it is interesting to note that more recent measurements made by this same group (21) indicate that the fluorescence cross section for a single blue-green algal species (anacystis marinus) was, on the average, 5 times larger than values obtained from monocultures representing the other three (red, golden brown and green) algal color groups. Assuming that the different algal color groups have similar fluorescence cross sections at 622 nm, it is not difficult to see how the summed effect of all other algal color groups will be sufficient to degrade any possible correspondence between the laser-induced fluorescence intensity and the concentration of blue-green algae even though the blue-greens were the dominant type over much of the flight line (see Figure 14). Remote monitoring of blue-green algae is further complicated by the fact that during non-bloom conditions, blue-greens are known to congregate below the surface layer. In other studies performed on Lake Mead (43), it has been noted that the concentration of blue-green algae tends to reach a maximum at depths below the 1-m surface layer. As the attenuation coefficients for the surface water along the flight line change from values less than 1 $m^{-1}$ to values close to 10 $m^{-1}$ between sampling

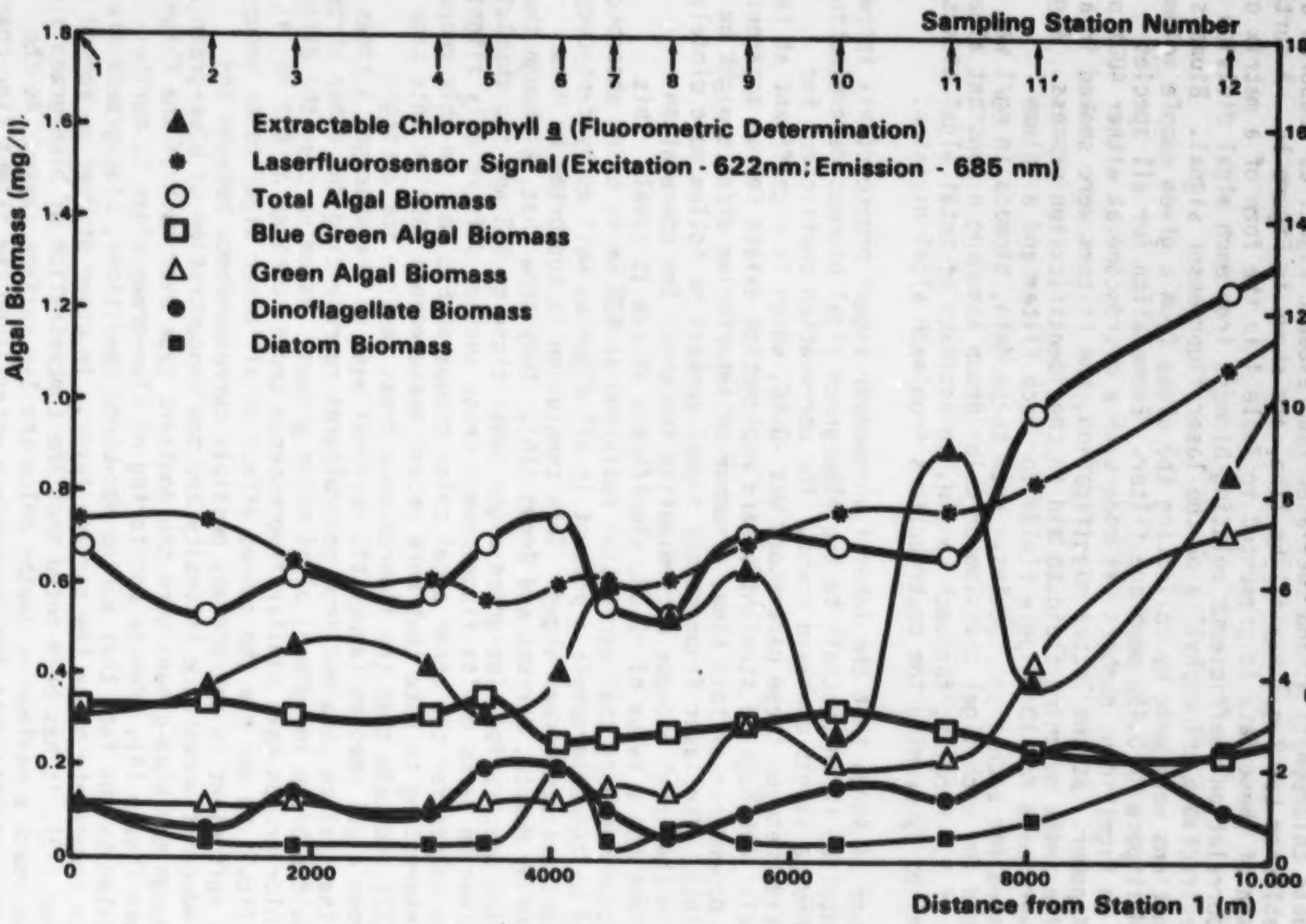

Figure 14. Variation of laser-fluorosensor signal, extractable chlorophyll a, total biomass and algal color group biomass with distance for surface transect of Las Vegas Bay in Lake Mead, Nevada. Flight #12, August 16, 1977.

### TABLE 4. CORRELATION COEFFICIENT MATRIX FOR ALGAL BIOMASS OBTAINED BY ENUMERATION VERSUS LASER-FLUOROSENSOR SIGNAL AND EXTRACTED CHLOROPHYLL a FOR A GROUP OF 13 SAMPLES

| Chlorophyll a Indicator<br>Biomass by Algal Division (mg/l) | Airborne Laserfluorosensor Signal [A] | Extracted Chlorophyll a [B,C] |
|---|---|---|
| Cyanophyta - Blue Greens (BG) | -0.48 | 0.57 |
| Chlorophyta - Greens (G) | 0.88 | 0.51 |
| Bacillariophyta - Diatoms (Dia) | 0.54 | 0.23 |
| Pyrrophyta - Dinoflagellates (Dino) | -0.08 | 0.36 |
| BG + G | 0.90 | 0.43 |
| BG + G + Dia | 0.89 | 0.42 |
| BG + G + Dino | 0.81 | 0.29 |
| Total Count | 0.82 | 0.31 |

A) Chlorophyll a fluorescence emission at 685 nm excited at 622 nm.

B) Fluorometric determination of extracted chlorophyll a using method of Holm-Hansen et al. (39).

C) Correlation between chlorophyll a and laserfluorosensor signal gave coefficient of 0.408.

situations 1 and 12, it is possible that the presence of blue-green algae is being progressively screened from the laser fluorosensor by increasing concentrations of the intervening material in the surface layer, in particular algae from other color groups. This explanation assumes that the depth at which the blue-green algae congregate remains a constant regardless of ambient conditions. In reality, however, this situation will be complicated by the tendency of the algae to adjust their depth according to the available light level compounded with the effects of other environmental factors, such as water temperature and availability of nutrients and dissolved oxygen.

It is interesting to note that extractable chlorophyll *a* exhibited a poor correlation with both total biomass (r = 0.31) and the laser fluorosensor signal (r = 0.41). The first result is somewhat surprising, as both the algae counts and the chlorophyll *a* extractions were performed on subsamples of the same grab sample material. More disturbing is the fact that chlorophyll *a* determinations are employed principally as a substitute or indicator for total algal biomass. In the light of these correlations between the laser fluorosensor signal and total algal biomass (r = 0.82), it is suggested that errors in the chlorophyll *a* determinations are the principal source of this discrepancy. As discussed in Section 7 (e), the established procedure for extracting chlorophyll *a* from algae is both inefficient and unreliable in terms of reproducibility. The large deviations in the extractable chlorophyll *a* profile in Figure 14 are considered to be due primarily to this problem. Improved methods for extracting chlorophyll *a* from algae are presently under investigation and will be reported elsewhere. In contrast to the spectrophotometric approach of Strickland and Parsons (38), the fluorometric method of Holm-Hansen et al. (39), used to make the determinations for this flight, does not correct for the fluorescence contributions from the chlorophyll *b* or carotenoid pigments. It is therefore planned to employ the spectrophotometric technique for establishing extractable chlorophyll *a* values in all future ground truth surveys. Because of this uncertainty in the accuracy of the chlorophyll *a* determinations for this flight and its effect on the correlation between the airborne and ground truth data, it has not been possible to establish the efficacy of using the 622-nm excitation wavelength for monitoring chlorophyll *a* in vivo. Future tests will therefore be conducted at a number of excitation wavelengths, in particular at 622 nm and 470 nm. The latter wavelength, although not responding to the pigments specific to red and blue-green algae, should provide a better chlorophyll *a* fluorescence response to the chlorophyll *b* and carotene pigments present in most algae than the original 440-nm excitation wavelength.

## SECTION 8

## AIRBORNE MEASUREMENTS OF THE INFLUENCE OF THE ATTENUATION COEFFICIENTS

### THEORETICAL CONSIDERATIONS

A relative measure of the variation in the diffuse attenuation coefficient $k_\lambda$ for surface waters, and consequently its dependence on $n_C$, can be obtained by monitoring the $\{OH\}$ stretch water Raman emission at wavelength R excited at laser wavelength L, concurrently with the measurement of chlorophyll a fluorescence emission at wavelength F. The wavelength of the Raman emission is determined by the excitation wavelength and the Raman frequency shift. For example, with laser excitation at 440 nm and a fixed $\{OH\}$ stretch water Raman frequency shift of 3,418 cm-$^1$, the Raman emission occurs at 518 nm. The intensity of the backscattered Raman emission signal will depend on the values of $k_L$ and $k_R$ and not on $n_W$, the concentration of water, which remains essentially constant. A laser fluorosensor equation for the detected Raman emission power $P_R$ can be written in a form analogous to the expression for the fluorescence power $P_F$ obtained from Equation 1 such that

$$P_R = \left(\frac{P_L}{H^2}\right) \frac{n_W \sigma_W d_R}{(k_R + k_L)} \tag{15}$$

where $\sigma_W$ is the Raman emission cross section for the OH stretch vibrational mode of liquid water. $\sigma_W$ is weakly dependent on temperature (44) and salinity (45) and exhibits a $1/\lambda^4$ dependence on the excitation wavelength, but, for a fixed laser wavelengtn and over a limited temperature range (5°C to 25°C) in fresh waters, it is assumed to remain constant. $d_R$ is dependent on the known system and environmental factors and is given by the expression

$$d_R = \left[\frac{T\eta_{Tr}\eta_{Rec}}{4\pi\Delta_R}\right] \left[\frac{(1-R_W)^2}{\mu_W{}^2 \exp\{H(\beta_L + \beta_R)\}}\right] \tag{16}$$

where $\Delta_R$ is that fraction of the Raman band seen by the detector and $\beta_R$ is the atmospheric attenuation coefficient at the Raman wavelength. All other terms are as defined for Equation 1. For clear atmospheric conditions over paths on the order of 300 m, the term $\exp\{H(\beta_L + \beta_R)\}$ is assumed to have a value that remains close to unity, so that $d_R$ is essentially a constant.

A similar expression for the received fluorescence power is obtained by rearranging Equation 1 such that

$$P_F = \left(\frac{P_L}{H^2}\right) \frac{n_C\,\sigma_C\,d_F}{(k_F + k_L)} \tag{17}$$

where

$$d_F = \left[\frac{T\eta_{Tr}\eta_{Rec}}{4\pi\Delta_F}\right] \left[\frac{(1-R_W)^2}{\mu_W{}^2 \exp\left\{H(\beta_L + \beta_F)\right\}}\right] \tag{18}$$

and is considered to be constant for the same reasons as given for $d_R$. An expression can now be written for the ratio $P_F/P_R$ using Equations 15 and 17, so that

$$\left(\frac{P_F}{P_R}\right) = \frac{n_C\,\sigma_C\,d_F}{n_W\,\sigma_W\,d_R} \left(\frac{k_R + k_L}{k_F + k_L}\right) \tag{19}$$

As $n_W$, $\sigma_W$, $\sigma_C$, $d_R$ and $d_F$ are all considered to be constant, a new expression can be written for $P_F/P_R$ in which

$$\left(\frac{P_F}{P_R}\right) = n_C\,\delta \left(\frac{k_L + k_R}{k_L + k_F}\right) \tag{20}$$

where $\delta = \left(\frac{d_F\,\sigma_C}{d_R\,n_W\,\sigma_W}\right)$ is a constant

The ratio $P_F/P_R$ is now seen to be dependent on $n_C$ and the ratio $(k_L + k_R)/(k_L + k_F)$ but is independent of the laser power $P_L$ and aircraft altitude H. However, care must still be taken to minimize H and maximize $P_L$ to ensure a good signal-to-background noise-ratio in the measurement of $P_F/P_R$. The question now arises as to the dependence of the ratio $(k_L + k_R)/(k_L + k_F)$ on changes in algae concentration and other dissolved and particulate matter in the surface waters. As changes in both $n_C$ and the concentration of the other substances will induce changes in all the $k_\lambda$ values simultaneously with the exact magnitude of the change depending on the specific wavelength, it is suggested that this ratio will exhibit only a weak dependence on $n_C$ or on the concentration of non-chlorophyllous materials. Consequently, if $(k_L + k_R)/(k_L + k_F)$ is assumed to be constant, then $P_F/P_R$ will vary simply as $n_C$.

A significant advantage of this approach to monitoring the degree of penetration of the laser radiation into the surface water is that it is independent of the theoretical model employed to describe the attenuation coefficients used in the laser-fluorosensor equation. In reality, the Raman radiation will be subject to the same physical constraints as the fluorescence radiation except for the effects due to the difference in wavelength. Clearly it would be advantageous to employ an excitation wavelength which shifts the water Raman emission band closer to the chlorophyll a fluorescence emission band at 685 nm.

It is therefore proposed to modify the airborne-laser fluorosensor described in Section 6 so that the water Raman signal can be detected, monitored and recorded concurrently with the fluorescence signal $P_F$, which will still be corrected for variations in $P_L$ and H. The ratio $P_F/P_R$ will then be correlated with chlorophyll a ground truth data. An improved correlation between $P_F/P_R$ and the chlorophyll a ground truth data, rather than with $P_F$ alone, will justify the assumption that the ratio $(k_L + k_R)/(k_L + k_F)$ remains essentially constant.

## LABORATORY MEASUREMENT OF FLUORESCENCE TO RAMAN RATIO FOR LAKE WATER SAMPLES

Before adding a Raman emission detection channel to the existing laser fluorosensor, it was considered expedient to conduct a laboratory experiment to simulate the airborne measurement of the ratio $P_F/P_R$ and in particular to ensure that $P_R$, the Raman emission power, could be measured with the same degree of sensitivity as the chlorophyll a fluorescence power, $P_F$. Liquid water consists of two spectroscopically distinct forms in thermal equilibrium. These polymeric and monomeric types influence the $\{OH\}$ molecular band strength and ultimately the intensity, spectral shape and polarization state of the $\{OH\}$ stretch Raman emission (45). In particular, the spectral characteristics of the Raman emission are highly dependent on the state of polarization of the excitation radiation, the scattering configuration used to view the emission and the polarization state of the detection system (46). The airborne laser-fluorosensor, of necessity, operates in a downward-looking mode which constitutes a 180° scattering angle with a plane-polarized laser source. Consequently, because of the dependence of the Raman band depolarization ratio on the scattering configuration, the state of polarization of the laser output, and the polarization sensitivity of the detection system, it is essential that the laboratory measurements simulate the airborne measurement as closely as possible. A plane-polarized laser was therefore employed as the excitation source, and the Raman radiation viewed in the 0° scattering configuration. The 0° scattering configuration, which is spectroscopically equivalent to a 180° format, was found to be more convenient to implement in a laboratory experiment. Finally, precautions were taken to ensure that the polarization sensitivity of the grating monochromator did not introduce errors into the measurement of the $P_F/P_R$ ratio. This step was accomplished by depolarizing both the fluorescence and Raman emission prior to transmission through the monochromator. This procedure was found to be necessary because spectral discrimination in the airborne system is performed with interference filters at normal incidence, which are not sensitive to the state of polarization of the incident radiation.

An optical schematic of this laboratory-mounted system is shown in Figure 15. A nitrogen laser pumped dye laser (AVCO Model 3000A), operating at 440 nm and, at a repetition rate of 500 Hz, was used to irradiate the lake water samples. This laser output has a pulse width of approximately 5 nsec with a spectral bandwidth of about 0.2 nm and is linearly polarized to within 1 part per 1,000. The stability of the laser output power was monitored with the use

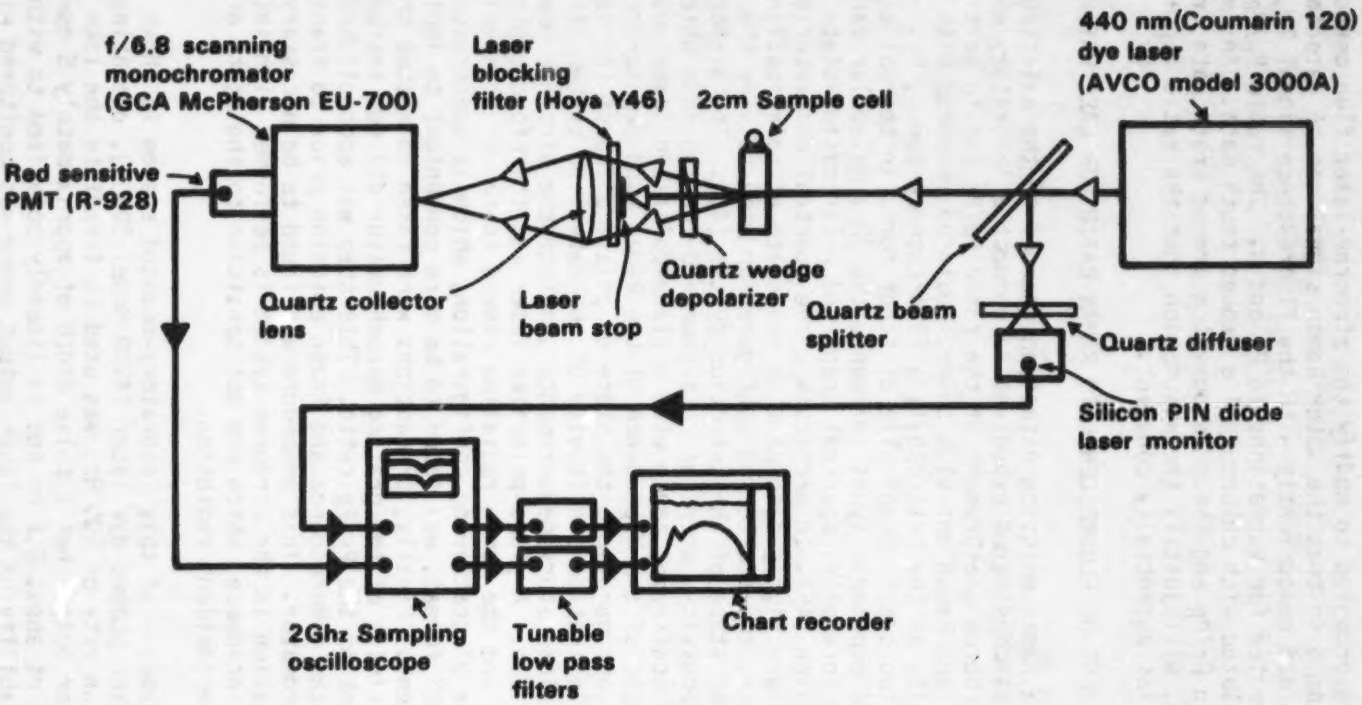
440 nm(Coumarin 120)
dye laser
(AVCO model 3000A)
Quartz beam
splitter
Quartz diffuser
Silicon PIN diode
laser monitor
2cm Sample cell
Quartz wedge
depolarizer
Laser
blocking
filter (Hoya Y46)
Laser
beam stop
Quartz collector
lens
f/6.8 scanning
monochromator
(GCA McPherson EU-700)
Red sensitive
PMT (R-928)
2Ghz Sampling
oscilloscope
Tunable
low pass
filters
Chart recorder

Figure 15. Laboratory simulation of airborne laser fluorosensor for monitoring chlorophyll a fluorescence and water Raman signals.

of a beam splitter to deflect a small percentage of the beam onto a PIN silicon photodiode via a quartz diffuser. The fluorescence and Raman emissions from the 2-cm pathlength cell were collected and focused onto the entrance slit of an f/6.8 scanning monochromator. A compensated, quartz wedge depolarizer was used to depolarize the fluorescence and particularly the Raman emission with a view to avoiding polarization artifacts being introduced into the ratio $P_F/P_R$ by the polarization sensitive grating monochromator.

Due to use of the 180° scattering configuration, care had to be taken to ensure that laser radiation did not reach the detector. In addition to the spectral discrimination provided by the single monochromator, a short wavelength cutoff glass filter (Hoya Y46) was employed to block wavelengths shorter than 460 nm. Further laser blocking was achieved by placing a small piece of black tape in front of the filter on the laser beam axis as shown in Figure 15. A fast, high-gain, red-sensitive photomultiplier (Hamamatsu R928), located close to the monochromator exit slit, was used to detect the fluorescence and Raman signals. Fluorescence and Raman bands of interest were obtained by scanning the monochromator over the spectral region of interest while monitoring the peak value of the emission pulse detected by the photomultiplier. The amplitude of this pulse was measured using a sampling oscilloscope (Philips PM 3400) operating in the non-scanning mode. The calibrated DC output signal from the oscilloscope sample and hold circuit, a measure of the peak amplitude of the detected pulse, was low-pass filtered to reduce photon noise effects and then plotted out directly as a function of wavelength using a strip chart recorder. The monochromator slit width and scanning rate, the low pass filter cutoff frequency, and the chart recorder speed were all adjusted so as to ensure that the narrowest spectral feature could be faithfully resolved. The $\{OH\}$ stretch water Raman band, which is located at 518 nm when excited at 440 nm has an established FWHM bandwidth of 11.5 nm at this wavelength and consequently is an ideal source for this purpose.

The spectra for two Las Vegas Bay surface water samples collected on August 16, 1977 and March 31, 1978 are shown in Figures 16 and 17 respectively. The respective chlorophyll a levels were 8.4 μg/l and 3.4 μg/l. It is clear, even from these rather noisy spectra, that, for the chlorophyll a range of interest (about 0.1 μg/l to 50 μg/l), the Raman band will provide a large and measurable signal for the airborne laser fluorosensor in relation to the chlorophyll a signal level as represented by the fluorescence pulse shown in Figure 7 for a chlorophyll a level of 10.5 μg/l.

Two points are worthy of note with regard to these spectra. First they are uncorrected for the varying spectral sensitivity of the grating monochromator and the photomultiplier. Both components have lower sensitivities in the red region at 685 nm than in the blue region at 518 nm. The blue-to-red sensitivity ratio for the R928 photomultiplier is 1.71 to 1, whereas that for the grating monochromator is approximately 1.25 to 1, giving a total ratio of 2.14 to 1. However, as the C31000A photomultiplier used in the airborne laser-fluorosensor has a blue-to-red sensitivity ratio of approximately 2.75 to 1, it is clear that the airborne measurement of the water Raman signal will be further enhanced in relation to the chlorophyll a fluorescence signal. The second point concerns the fluorescence background

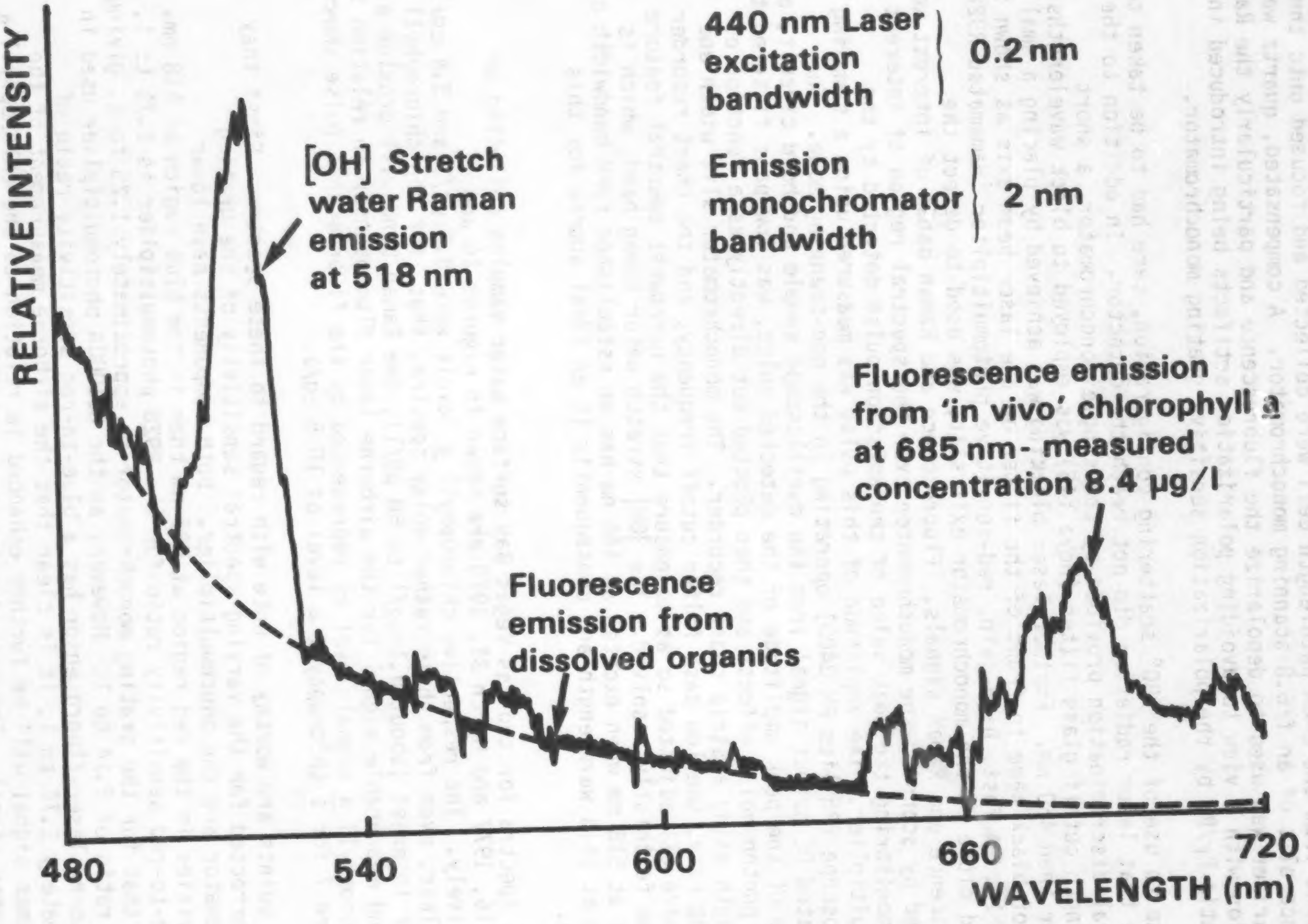

Figure 16. Uncorrected fluorescence and Raman emission spectra of Lake Mead surface water sample, excited at 440 nm, August 16, 1977.

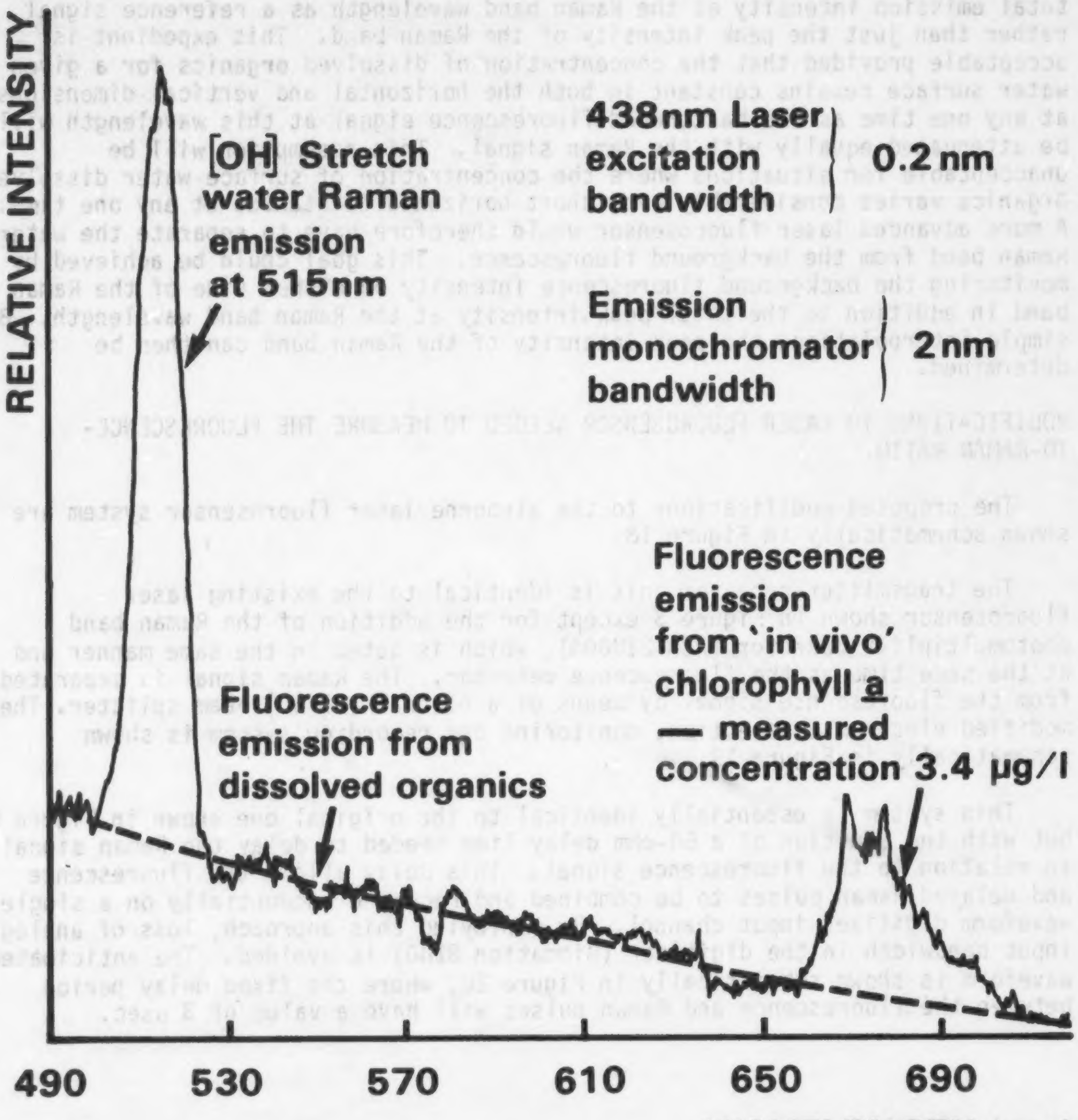

Figure 17. Uncorrected fluorescence and Raman emission spectra of Lake Mead surface water sample, excited at 438 nm, March 31, 1978.

due to dissolved organics, indicated in Figures 16 and 17 by broken curves. This background signal, which has a fluorescence emission maximum in the region of 430 nm, constitutes only a small fraction of the total signal at 685 nm for Lake Mead surface water and, as such, its influence on the chlorophyll a fluorescence measurement can be neglected. In addition this background fluorescence signal has been shown to remain fairly constant over the extent of Lake Mead at any one time (47). The present plan is therefore to use the total emission intensity at the Raman band wavelength as a reference signal rather than just the peak intensity of the Raman band. This expedient is acceptable provided that the concentration of dissolved organics for a given water surface remains constant in both the horizontal and vertical dimensions at any one time as the background fluorescence signal at this wavelength will be attenuated equally with the Raman signal. This assumption will be unacceptable for situations where the concentration of surface-water dissolved organics varies considerably over short horizontal distances at any one time. A more advanced laser fluorosensor would therefore have to separate the water Raman band from the background fluorescence. This goal could be achieved by monitoring the background fluorescence intensity on either side of the Raman band in addition to the total peak intensity at the Raman band wavelength. By simple interpolation, the peak intensity of the Raman band can then be determined.

## MODIFICATIONS TO LASER FLUOROSENSOR NEEDED TO MEASURE THE FLUORESCENCE-TO-RAMAN RATIO

The proposed modifications to the airborne laser fluorosensor system are shown schematically in Figure 18.

The transmitter-receiver unit is identical to the existing laser fluorosensor shown in Figure 3 except for the addition of the Raman band photomultiplier detector (RCA 31000A), which is gated in the same manner and at the same time as the fluorescence detector. The Raman signal is separated from the fluorescence signal by means of a neutral (50 50) beam splitter. The modified electronic detection, monitoring and recording system is shown schematically in Figure 19.

This system is essentially identical to the original one shown in Figure 6 but with the addition of a 50-ohm delay line needed to delay the Raman signal in relation to the fluorescence signal. This delay allows the fluorescence and delayed Raman pulses to be combined and recorded sequentially on a single waveform digitizer input channel. By employing this approach, loss of analog input bandwidth in the digitizer (Biomation 8100) is avoided. The anticipated waveform is shown schematically in Figure 20, where the fixed delay period between the fluorescence and Raman pulses will have a value of 3 μsec.

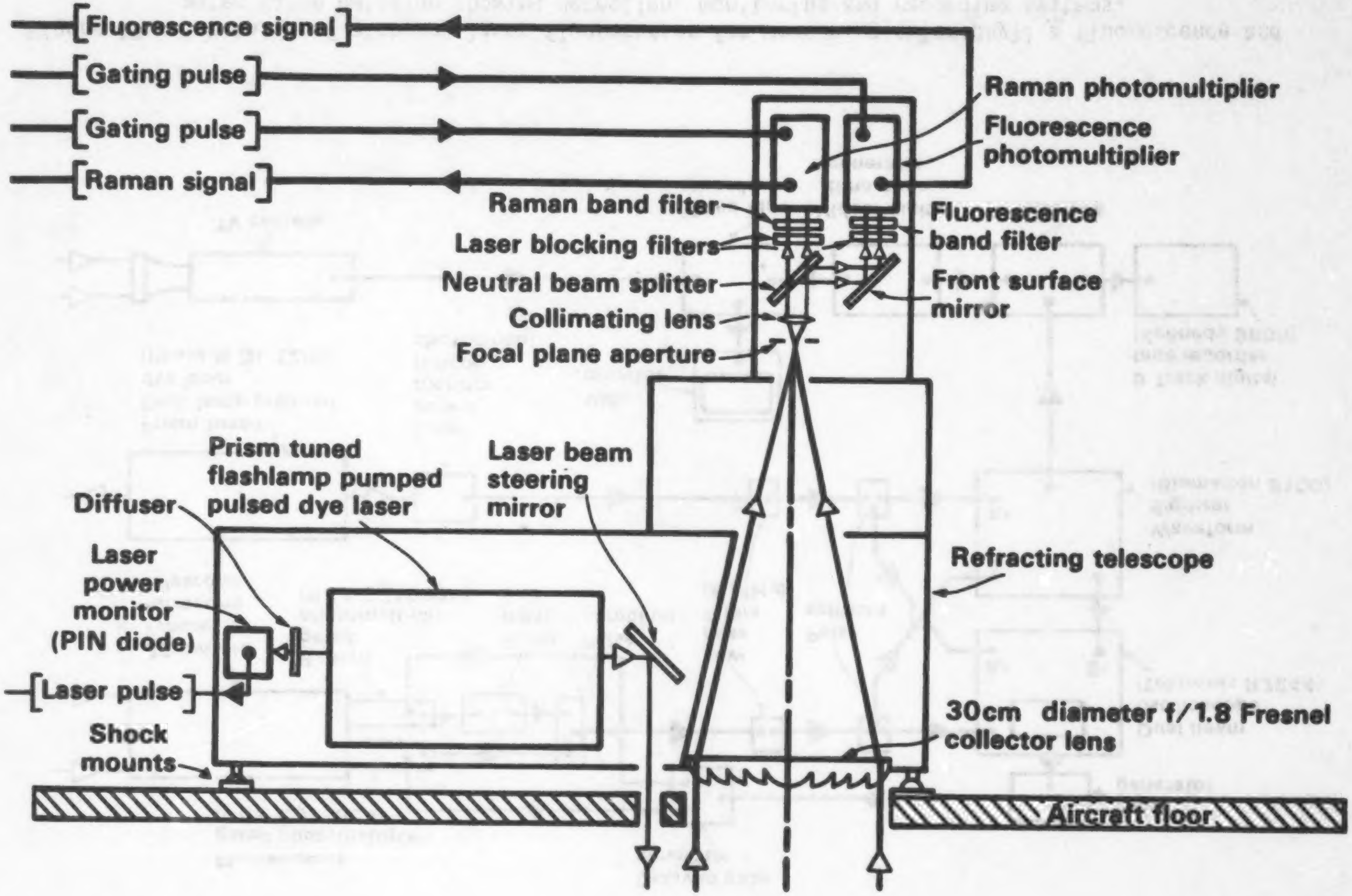
Fluorescence signal
Gating pulse
Gating pulse
Raman signal
Raman photomultiplier
Fluorescence photomultiplier
Raman band filter
Laser blocking filters
Neutral beam splitter
Collimating lens
Focal plane aperture
Fluorescence band filter
Front surface mirror
Prism tuned flashlamp pumped pulsed dye laser
Laser beam steering mirror
Diffuser
Laser power monitor (PIN diode)
Laser pulse
Shock mounts
Refracting telescope
30cm diameter f/1.8 Fresnel collector lens
Aircraft floor

Figure 18. Optical diagram of airborne laser fluorosensor for monitoring chlorophyll a fluorescence and water Raman signals.

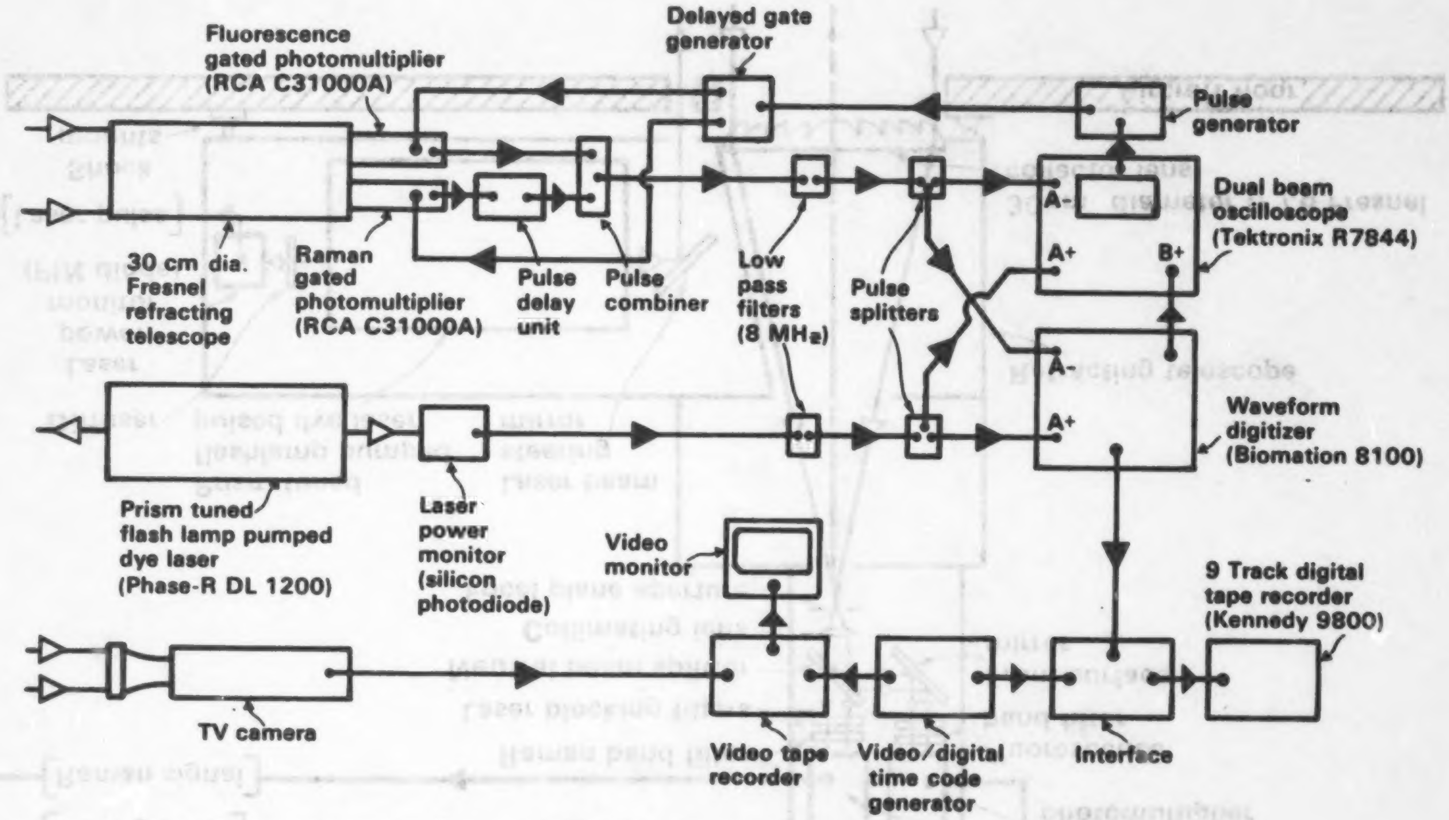
Fluorescence gated photomultiplier (RCA C31000A)
Delayed gate generator
Pulse generator
Dual beam oscilloscope (Tektronix R7844)
A-
A+
B+
30 cm dia. Fresnel refracting telescope
Raman gated photomultiplier (RCA C31000A)
Pulse delay unit
Pulse combiner
Low pass filters (8 MHz)
Pulse splitters
A-
A+
Waveform digitizer (Biomation 8100)
Prism tuned flash lamp pumped dye laser (Phase-R DL 1200)
Laser power monitor (silicon photodiode)
Video monitor
9 Track digital tape recorder (Kennedy 9800)
TV camera
Video tape recorder
Video/digital time code generator
Interface

Figure 19. Schematic of airborne laser fluorosensor for measuring chlorophyll a fluorescence and water Raman emission showing detection, monitoring and recording systems.

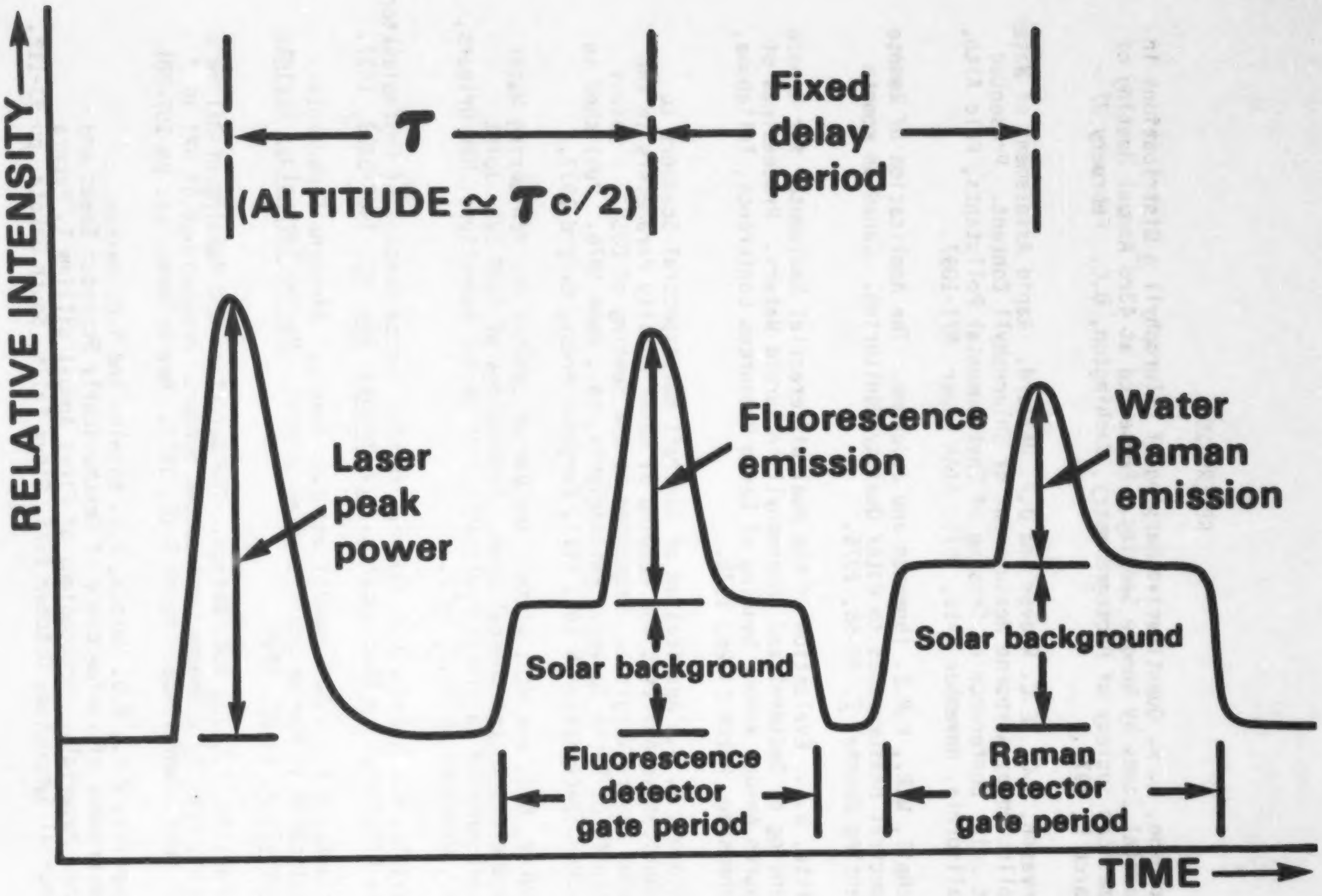
RELATIVE INTENSITY
T
(ALTITUDE ≃ Tc/2)
Fixed delay period
Laser peak power
Fluorescence emission
Water Raman emission
Solar background
Solar background
Fluorescence detector gate period
Raman detector gate period
TIME

Figure 20. Variation of laser, fluorescence and Raman laser-fluorosensor signals as a function of time.

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